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Dear Pharmaceutical Chemist:

This issue of Waters Pharmaceutical Notes contains articles on:

- Using a Coupled Achiral-Chiral HPLC System to Determine the Mefloquine Enantiomers in Plasma and Whole Blood
- Applications of FLEC-Derivatizing Agent for Chiral Separation and Fluorescence Detection
- Capillary Electrophoresis of Small Molecule Pharmaceuticals
- System Suitability Software for Increasing Laboratory Productivity
- Analysis of Inorganic Ions in a Vitamin Tablet

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We look forward to hearing from you.

Sincerely,

Peter C. Rahn

Peter C. Rahn, Ph.D.
Pharmaceutical Marketing Manager

PHARMACEUTICAL NOTES

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Using a Coupled Achiral-Chiral HPLC System to Determine the Mefloquine Enantiomers in Plasma and Whole Blood.

by Francois Gimenez and Irving W. Wainer,* Pharmaceutical Division, McGill University, Montreal, Canada

Introduction.

The stereoisomeric composition of drug substances is rapidly becoming a key issue in the development, regulation and administration of pharmaceuticals. This concern has centered on the enantiomeric composition of these substances, and has been spurred on by the recognition that the enantiomers of a pharmacologically active compound often differ in their efficacy, toxicity and pharmacological disposition.

The most widely used analytical technique for the separation and quantitation of enantiomers is HPLC. The separation of enantiomers is a significant HPLC challenge because the physical and chemical properties of the two optical isomers are identical. Typical chiral analysis approaches include the use of specialty chiral columns, chiral mobile phase modifiers and derivatization using homochiral reagents. When enantiomers must also be resolved from other non-chiral compounds the HPLC analysis becomes even more difficult. To solve this difficult problem a coupled achiral-chiral HPLC system has been developed. This technique has been used to determine the mefloquine (MFQ) enantiomers in plasma and whole blood.

Mefloquine is a chiral molecule which is used for the treatment and prophylaxis of malaria. The agent is administered orally as a racemic mixture of the (+) and (–) forms, Figure 1. Initial studies of MFQ pharmacokinetics have demonstrated that the drug has a long half-life, but these studies did not consider the plasma disposition and elimination of the MFQ enantiomers. The potential importance of determining the pharmacological fate of the separate MFQ enantiomers was indicated by a study which found that (–) MFQ is a more potent acetylcholinesterase and butylcholinesterase inhibitor than (+) MFQ[1]. In addition, there have been conflicting studies regarding differential accumulation of MFQ in erythrocytes versus plasma[2,3]. The enantioselectivity of this accumulation had not been studied.

The initial separation (non-enantiomeric separation) was performed on an achiral column. The total quantity of (+) and (–) MFQ was determined on the cyano bonded phase column and any interfering components in the biological matrix were removed during this first separation. The enantiomeric composition [(+) and (–) MFQ ratio] was determined on the (S)-naphthylurea chiral stationary phase. The two columns were connected by a switching valve equipped with a silica precolumn. The precolumn concentrated the MFQ in the eluent from the achiral column before backflushing onto the chiral phase. Although this coupled column system was specifically validated and applied to the pharmacokinetic analysis pilot study for the (+) and (–) MFQ concentration in plasma and whole blood, the technique can readily be applied for many other chiral compounds where matrix affects are a major concern and sensitivity is a major issue.

IN THIS ISSUE

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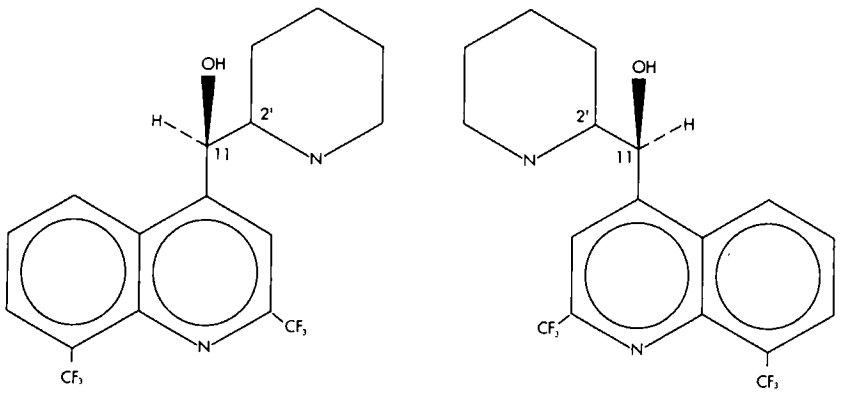
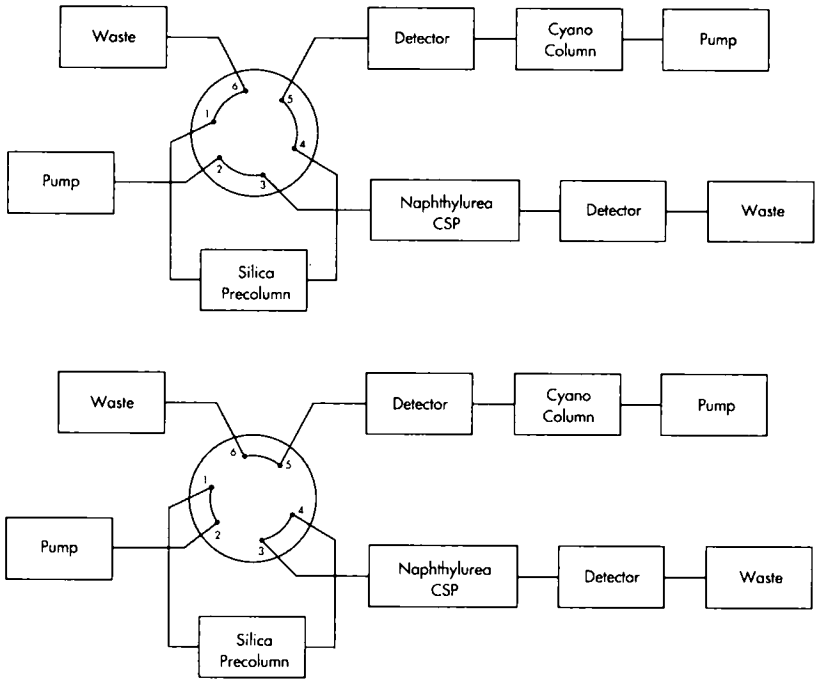
Figure 1: Structure of Mefloquine Enantiomers.



The enantiomers of MFQ can be directly resolved on a (S)-naphthylurea chiral stationary phase using a hexane: 2-propanol: methanol mobile phase with a stereoselectivity (α) factor of 1.63. Due to interferences from endogenous compounds in plasma and whole blood samples, this separation could not be directly applied. To overcome this problem, a coupled achiral - chiral HPLC system was developed (shown in Figure 2) which minimized the analysis time while requiring only one sample injection.

Figure 2: The Achiral-Chiral Coupled Column HPLC System Minimizes Analysis Time.



Waters 600E Multisolvent Delivery System delivers the mobile phase for the cyano column. The high backpressure capabilities of the Waters 484 Tunable Absorbance Detector and minimal bandspreading within the detector cell allow in-line coupling to a second system providing simultaneous assay and chirality measurements.

Apparatus.

The analytical method involved two chromatographic systems connected through a Rheodyne Model 7010 switching valve equipped with a 10 µm silica gel column. The achiral separation was performed with a Waters 600E Multisolvent Delivery System, a manual injector, and a Waters 484 Tunable Absorbance Detector set at 285 nm. The 484 tunable UV detector cell could withstand the back pressure from the second chiral column and the band broadening was minimal allowing quantitation of the total MFQ and the enantiomers with a single injection. The MFQ was separated from interferences in the biological matrix and quantitated on an achiral cyano bonded phase column using the mobile phase specified in Figure 3. By placing the 484 Tunable Absorbance Detector after the achiral column it was possible to detect when the MFQ enantiomers eluted from the achiral column. When this peak eluted from the achiral column it was concentrated on the small guard column and then backflushed to a second isocratic HPLC system using the switching valve. The second HPLC system consisted of an isocratic pump and a variable UV detector set at 285 nm. The stereochemical separation of (+) and (–) MFQ chiral separation was accomplished on a 5 µm (S)-naphthylurea stationary phase. Data from both coupled systems was collected on integrators.

Sample Preparation.

Collection of samples: Blood samples were collected from a healthy male Caucasian volunteer after administration of 15 mg/kg of MFQ base. Blood samples were collected before administration and at various intervals through 30 days. The samples were

separated after collection with 2 ml of whole blood transferred to one polypropylene tube while the remaining sample was centrifuged and the plasma collected and transferred to a separate polypropylene tube. All samples were stored at -20°C until analyzed.

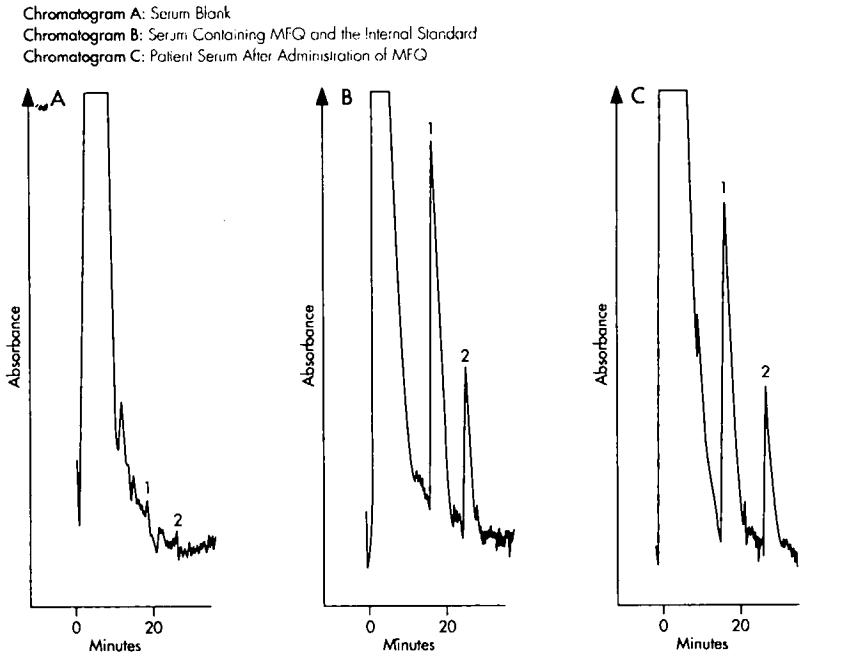
Extraction Procedure and Response Curves.

MFQ was extracted from whole blood and plasma according to Franssen, et. al.,[4]. The sample was concentrated by extraction and evaporation. Prior to injection the sample was dissolved in the mobile phase and analyzed. The recovery and reproducibility of this method was investigated using five drug free serum samples spiked between 0.1 µg/ml and 1.6 µg/ml racemic MFQ with chloroquine (CQ) added as the internal standard to each sample. A standard curve for total MFQ [i.e., (+) and (-) MFQ] plasma concentrations was prepared by the addition of 0.1 to 1.6 µg/ml to drug free serum. The total (+) and (-) MFQ study was carried out on the Waters 600E based chromatographic system with the achiral column and a standard curve was constructed by plotting the MFQ/CQ peak height ratios versus the known MFQ concentrations.

Results and Discussion.

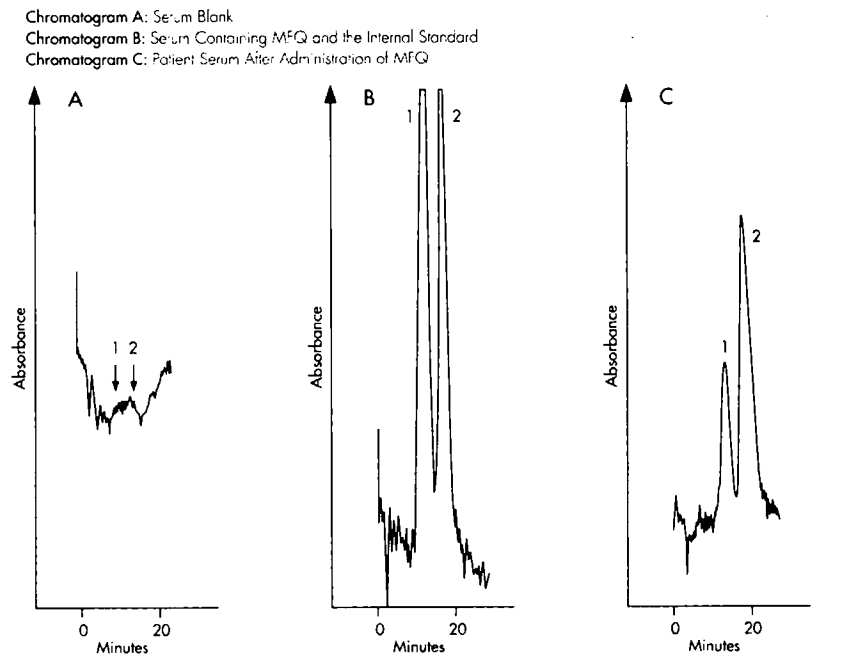
Under the achiral chromatographic conditions used in this study, the chromatographic retention times of MFQ and CQ were 7.6 and 12 minutes, respectively. The MFQ and CQ were well resolved from each other and from interfering compounds from the serum matrix (Figures 3 and 4).

Figure 3: MFQ Separation on an Achiral Cyano Bonded Phase Column.



The cyano column with the mobile phase hexane: 2-propanol: methanol (82:4:14) modified with 0.005% triethylamine separates any biological interferences from the internal standard (peak 2) and the (+, -) MFQ enantiomers (peak 1).

Figure 4: The MFQ Enantiomer Separation on the Chiral Stationary Phase After Initial Separation on the Achiral Cyano Column.



The enantiomer ratio is calculated after separation on the chiral stationary phase using a normal phase mobile phase. The (+) MFQ enantiomer (peak 1) elutes first, followed by the (-) MFQ enantiomer (peak 2).

The standard curve for the MFQ was linear over the range investigated with a correlation coefficient of 0.9993. The limit of detection was 0.05 µg/ml and the percent recovery ranged from 85% at the low concentrations (0.1 µg/ml) to 90% at the high concentrations (1.6 µg/ml).

Representative chromatograms of the MFQ enantiomer separation on the chiral stationary phase are presented in Figure 4. The k's for (+) MFQ and (–) MFQ were 7.9 and 12, respectively. Since baseline resolution of the MFQ enantiomers was not obtained under the chromatographic conditions used in this study, the (+) and (–) MFQ peak height ratio was investigated over the expected plasma concentration range. The observed ratio of 1.1 was used as a correction factor in the calculation of the actual percentage composition of each enantiomer.

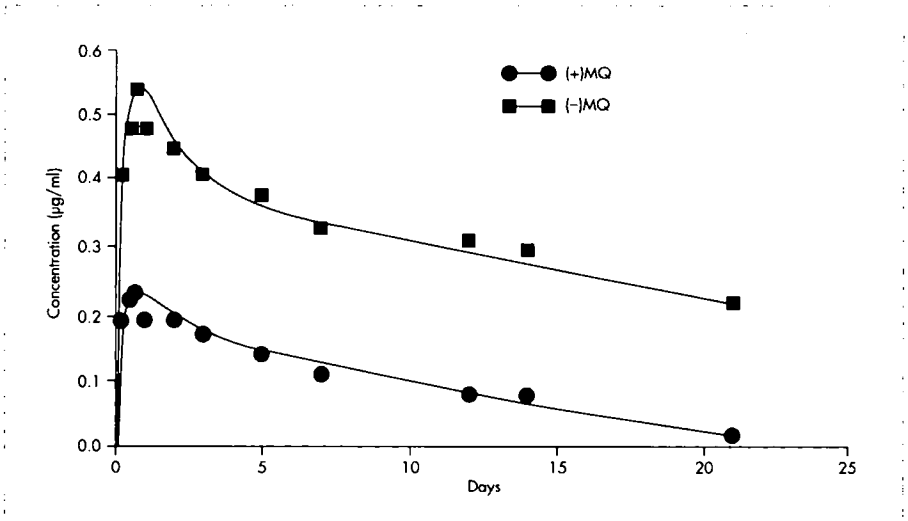
Pharmacokinetic Pilot Study.

The total MFQ concentration was determined on the achiral section of the coupled chromatographic system and the percentages of (+) MFQ and (–) MFQ were determined on the chiral section. Both determinations were carried out in a single experiment with one sample injection minimizing operator time, calculations and correlations.

The plasma concentration versus time curves for the enantiomers of MFQ are presented in Figure 5. The initial results indicate that both the absorption and elimination of the drug are stereospecific. At each experimental point, the plasma concentration of (–) MFQ enantiomer was greater than that of the (+) MFQ enantiomer. The MFQ plasma concentration ratio varied from 1.7 at two hours to 11.5 at 504 hours. Without the stereoselective coupled column chromatography an achiral assay would provide incorrect data on the disposition of this drug.

The whole blood concentration versus time curves for the enantiomers of MFQ also differed, but the magnitude of the difference between the two enantiomers was not as great. The (+) / (–) MFQ whole blood concentration ratio varied from 1.5 at 2 hours to 3 at 504 hours. Results of the complete pharmacokinetic study are not reported in this article.

Figure 5: Plasma Concentration Versus Time Curves for the Enantiomers of MFQ.



The MFQ was administered as a racemic mixture but the plasma levels differ significantly. To ensure accurate blood level measurements of the active enantiomer, the individual enantiomers must be determined.

Summary.

Chirality has become a major issue in recent years with much work ongoing to determine the enantiomeric ratio and purity of new drugs that contain chiral centers. In addition this work indicates that the pharmacokinetics and stereoselectivity of drug metabolism is of prime importance and cannot be neglected. As shown here, a method that couples achiral and chiral columns can provide added selectivity, specificity and sensitivity, allowing more accurate chiral analysis from challenging sample matrices.

Acknowledgments.

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For more information on Waters 600E Multisolvent Delivery System check box 4 on the attached reply card. For more information on Waters new 486 Tunable Absorbance Detector (replaces the 484 Detector) for coupled achiral-chiral analysis please check box 5 on the attached reply card.

Applications of FLEC - Derivatizing Agent for Chiral Separation with Fluorescence Detection.

by Peter C. Rahn, Pharmaceutical Marketing Manager

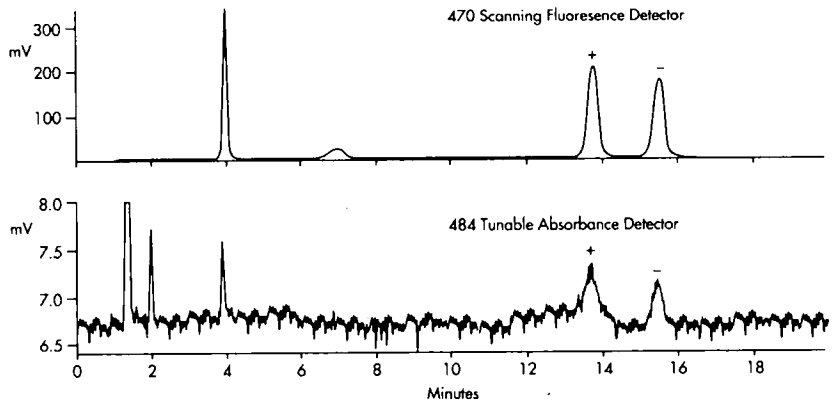
Waters 470 Scanning Fluoresence Detector Brings a New Dimension to Chiral Analysis.

Enantiomer specific analysis of optical isomers are needed in a variety of situations. These include the analysis of the enantiomeric composition of drugs, synthetic intermediates, monitoring stereospecific synthesis, or in pharmacological testing. In these situations, several approaches to the determination of enantiomers have been used. In recent work by J. Gal[1], derivatization was employed to form diastereomers from enantiomers. This indirect method separates the diastereomers using a typical reverse phase achiral HPLC column. The same indirect method can be used with capillary electrophoresis using non-chiral discriminating electrolytes. Work by I. Wainer[2] involves the direct analysis of enantiomers by a combination of chiral and achiral HPLC columns in pharmacokinetic studies. The total drug is measured by the achiral column and the enantiomeric ratio determined by the coupled chiral column. Another method being researched in Waters Pharmaceutical Laboratory is the use of capillary electrophoresis to separate the enantiomers without derivatization. An example of this work is presented elsewhere in this issue of pharmaceutical notes (page 7).

Each chiral separation technique has specific advantages depending on the sample matrix. The direct method employing chiral columns is often used for enantiomer analyses during chemical synthesis, stability studies or in the determination of a pharmacological agent before pharmacokinetic testing. In most cases the sample matrix is relatively clean (i.e., few interfering peaks are detected). Since chiral columns are custom designed for chiral discrimination, these are not as useful to separate non-chiral components, degradation products or drug metabolites.

Waters Pharmaceutical Laboratory has explored the analysis of chiral compounds by the indirect method to provide advantages over the current direct and indirect methods. The advantages of this new technique, using a fluorescent homochiral reagent and Waters 470 Scanning Fluoresence Detector, are enhanced sensitivity, selectivity and stability of the derivative. Although the choice of the homochiral derivatizing agent in the indirect method depends on the available enantiomer functional groups, this approach has been highly successful in a variety of stereospecific analysis problems.

Figure 1: Comparison of the UV and Fluorescence Response for the Derivatized Racemic Mixture of Ephedrine.



Waters 470 Fluorescence Detector provides over 2400 times more sensitivity for the derivatized sample compared to UV detection. The fluorescence detection limit for this derivative is 12 picograms.

Optimized Systems and Columns for Diastereomer Analysis.

The instrumentation used in this indirect method included a Waters 600E Multisolvent Delivery System, the 470 Scanning Fluorescence Detector, the 484 Tunable Absorbance Detector and the 715 Ultra WISP™ Sample Processor. All data was collected on a VAX™ based 845 Workstation. Derivatization was performed with (+) - 1 - (9-fluorenyl)ethylchloroformate (FLEC) as described by S. Einarsson[3]. The diastereomers were separated using the highly efficient 5 micron Nova-Pak® C_{18} columns in either steel or radial compression cartridge configurations. The mobile phase consisted of 20 mM monobasic ammonium phosphate buffer and various proportions of acetonitrile.

Fluorescence Detection Increases Sensitivity.

Once an appropriate separation was developed, each peak was scanned using the unique scanning capabilities of the Waters 470 detector to optimize the excitation and emission wavelengths. Enhanced sensitivity is a major advantage of the fluorescent FLEC derivative. In Figure 1, a derivatized racemic mixture of Ephedrine was injected and the separation monitored by both UV and fluorescence. The enhanced sensitivity provided by the FLEC derivative coupled with the high sensitivity of the 470 detector provides a 2400 fold increase in sensitivity over UV detection. The detection limit (signal to noise ratio of 3:1) for ephedrine using the UV detector was 5 nanograms while the detection limit was 12 picograms with the fluorescence detector. Not only does the FLEC derivative provide different selectivity, chiral discrimination and increased sensitivity, it imparts fluorescent activity to compounds not possessing native fluorescence, expanding the applicability of the fluorescent detector.

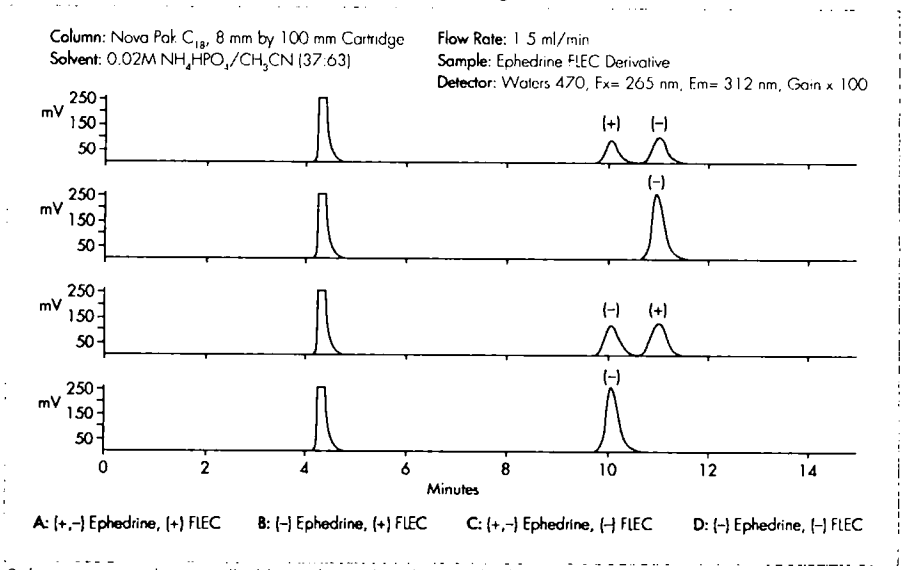
Diastersomer Stability Allows Overnight Analysis.

In order to study the stability of the FLEC derivative, derivatized samples were stored at room temperature, and chromatographed every hour during a 17 hour period. The peak area's relative standard deviation was 0.6% over this time period. No significant degradation of the derivatives was observed nor did the area response change under these conditions.

Minor Component Analysis Made Easy by Controlling Elution Order.

Another major advantage of the indirect method is that the elution order of the compound of interest can be easily altered. Figure 2 shows the separation of the ephedrine-FLEC diastereomers. Since FLEC is available in

Figure 2: Controlling Selectivity with a Homochiral Reagent.



Selectivity is easily controlled by judiciously selecting the homochiral derivatizing reagent. The derivatizing reagent is chosen to elute the least concentrated diastereomer first.

both the (+) and (−) forms the diastereomer elution order can be reversed by using the appropriate form of the reagent. This is important when assaying non-racemic enantiomer mixtures. When the enantiomer ratio differs by 100:1 or greater as occurs in pharmacokinetic studies or in chiral specific synthesis, the minor enantiomer quantitation can be difficult. Using the appropriate homochiral reagent the trace level enantiomer can always elute before the major enantiomer providing better integration and quantitation. This ensures good accuracy and precision even if the enantiomer ratio is 100:1 or greater. With the selectivity available for the FLEC derivative, it is easy to customize a method which provides excellent precision and accuracy.

Manual or Automated Derivatization Offered with Waters Sample Processor.

Whether to form the derivative manually or with the WISP 1700 Sample Processor is dictated by other sample matrix requirements. Based on Waters MilliLab™ Workstation, the WISP 1700 Sample Processor performs all operations involving handling liquids including automated filtration and solid phase extraction with a unique, streamlined probe on a rugged XYZ Transport Module. If biological samples need to be extracted, concentrated or cleaned prior to derivatization, then the WISP 1700 is the appropriate sample preparation tool.

Summary.

The use of FLEC derivatives with high sensitivity fluorescence detection brings a new dimension to chiral analyses. The demonstrated ease of use, versatility, enhanced sensitivity, precision and reliability provide the researcher with a new tool for enantiomer separations.

References:

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3. S. Ehrsson, Analytical Chemistry, 59, 1191, (1987)

For more information on Waters 470 Scanning Fluorescence Detector please check box 6 on the attached reply card. For more information on Waters WISP 1700 Sample Processor please check box 7 on the attached reply card.

Capillary Electrophoresis of Small Molecule Pharmaceuticals.

by Peter C. Rahn, Pharmaceutical Marketing Manager

Waters New Quanta™ 4000 Capillary Electrophoresis System Provides A New Separations Tool for the Pharmaceutical Chemist.

As pharmaceutical researchers develop more complex molecules and targeted drug formulations, it has been demonstrated that capillary electrophoresis can play an important role in the development of these new products. Capillary electrophoresis has many unique advantages – high efficiency, reproducibility, selectivity, broad pH range capability and fast analysis times while only using sample volumes from 1 to 10 nanoliters. Capillary electrophoresis separations generally offer unique selectivity, different from HPLC or GC.

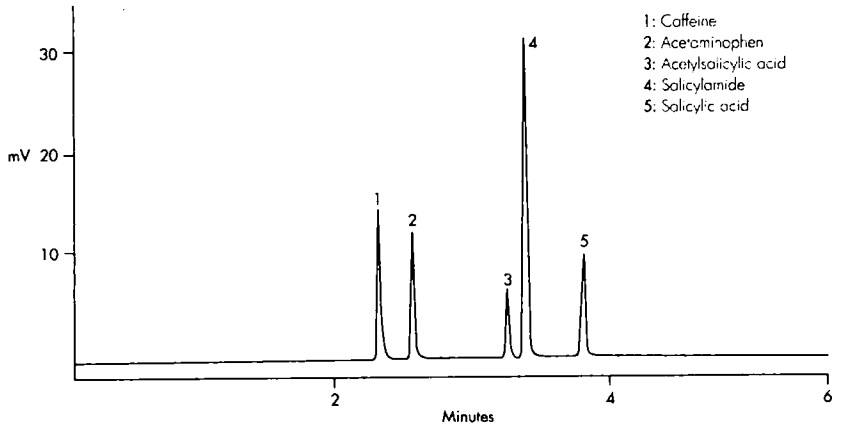
With the commercial availability of capillary electrophoresis, researchers now have available another reliable tool to help determine or confirm drug purity, dosage form degradation or perform chiral separations. Capillary electrophoresis can provide different selectivity from HPLC giving more information about a compound and can play an important role in the new product development process. Resolution, selectivity and analysis time can be optimized by altering applied voltage, buffer composition, ionic strength, pH, adding micelles, using gel filled capillaries, or by chemically modifying the capillary wall.

CE Generates Over 100,000 Theoretical Plates for Small Molecule Separations.

Capillary electrophoresis is a very powerful separation tool due to the large number of theoretical plates possible in the separation. The separation efficiency is directly related to the solute mobility and the voltage used. Thus, the higher the applied voltage, the higher the efficiency of the separation. Under normal operating conditions between 20 and 30 kilovolts, 100,000 to 200,000 theoretical plates can be routinely achieved for small pharmaceutical compounds with a 60 cm capillary.

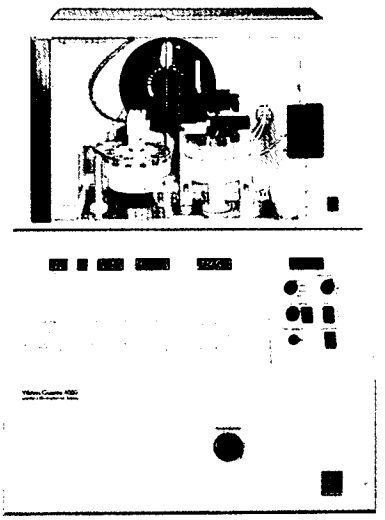
To demonstrate the separation power available with capillary electrophoresis, Waters Pharmaceutical Laboratory performed an analysis of common analgesics. In this experiment, the Quanta 4000 Capillary Electrophoresis System performed the analysis in less than four minutes (Figure 1). A similar HPLC separation requires 12 to 15 minutes. In addition to saving analysis time, this CE method generated in excess of 100,000 plates providing better separation and resolution. Although both HPLC and CE could perform this analgesic analysis, other assays may be easier to perform on CE.

Figure 1: The Quanta 4000 Capillary Electrophoresis System Separates Analgesics in Less Than Four Minutes.



Over 100,000 theoretical plates were generated for this four minute separation on a 60 cm x 75 µm i.d. capillary providing high resolution and very short analysis time.

Waters Quanta 4000 Capillary Electrophoresis System.



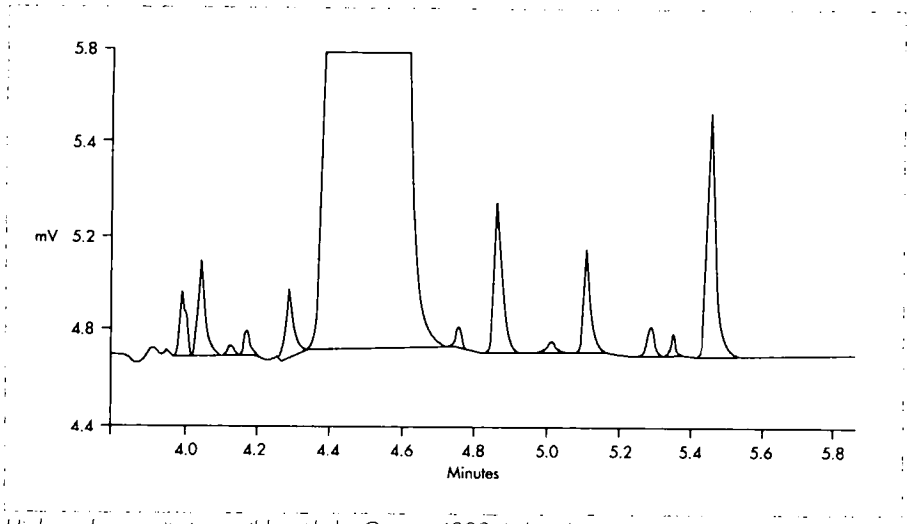
The Waters Quanta 4000 Capillary Electrophoresis System includes an autosampler, capillary, selectable UV/Vis detector and fraction collector in a single compact design

CE Provides High Sensitivity and High Peak Capacity with Short Analyses Times.

The advantage of very high efficiency and unique selectivity is illustrated in the capillary electrophoresis separation of a compound which appeared to be *pure*. This CE separation requires less than six minutes, but more importantly, thirteen peaks of interest were detected when the detector sensitivity was increased on the data system (Figure 2).

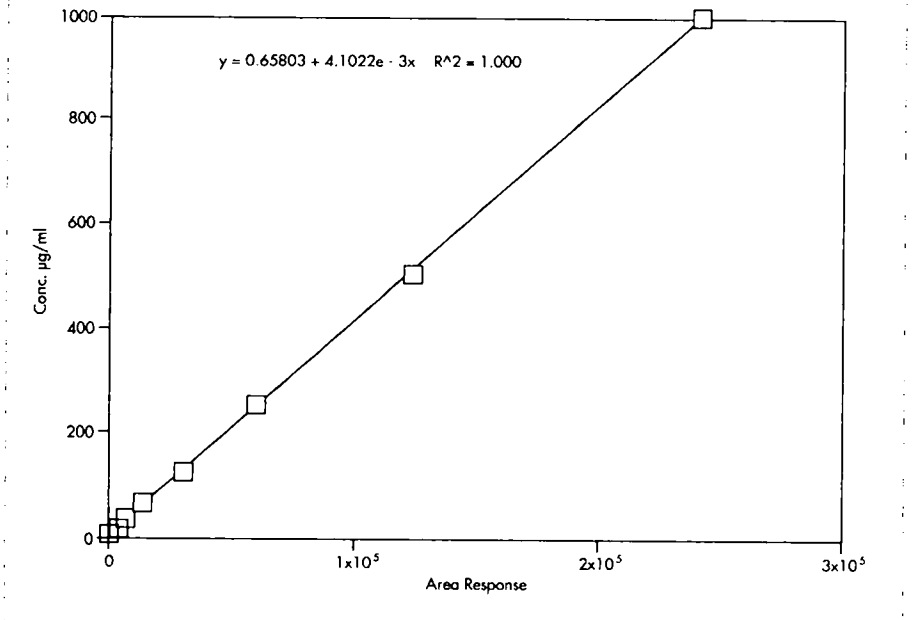
High peak capacity, the ability to separate a large number of closely related materials in a very short time, is a major capillary electrophoresis benefit. To quantitate these compounds the data was collected on a Waters 845 VAX based data station and the normalized area counts calculated. For this separation, the Quanta 4000 high sensitivity detector was able to measure peaks with 0.01% of the area count of the major compound. The selectivity and high efficiency allowed the total analysis time to be less than six minutes whereas a similar HPLC separation may require a 20 to 30 minute gradient method.

Figure 2: CE Separation of a Major Component and 12 Impurities.



High peak capacity is possible with the Quanta 4000. In less than two minutes 13 peaks are separated and quantitated. The smallest peaks are less than 0.1% of the major component.

Figure 3: Linear Calibration Curve for Salicylamide on the Quanta 4000 Capillary Electrophoresis System.



Laboratory productivity is increased since single point calibration curves are utilized when the correlation coefficient equals one.

Excellent Detector Linearity Over Three Orders of Magnitude.

Today, any new analytical technique must be reproducible and quantitative. A non-linear detector response would require multiple level calibration curves to be routinely generated, reducing the number of *real samples* which can be processed. A linear calibration plot dramatically reduces the number of standards required to recalibrate a method, allowing more *real* samples to be assayed during a work day. As shown by the linear calibration curve of the Quanta 4000 for salicylamide, routine analysis can be performed by periodically injecting single standards and updating the slope of a calibration equation (Figure 3). Excellent linearity was achieved for this separation with a correlation coefficient equal to 1.00 under these conditions. With a 75 µm i.d. capillary, the minimum sample concentration which produced a signal to noise level equal to three (S/N = 3) was 73 ng/ml.

HPLC and CE Accuracy, Precision and Reproducibility are Equivalent.

Routine analysis can be performed accurately and precisely with the Waters Quanta 4000. For an example, reproducibility was evaluated for the analgesic separation mentioned in Figure 1. The standard deviations for migration time and area counts for nine injections were less than 1% and 2%, respectively. Longer term system suitability testing applied to the CE separation over a fifteen-hour period reveals that the overall analysis trend for migration time was less than 1% and the overall area counts varied approximately 2%. The accuracy of a CE method in our laboratory has been equivalent to HPLC or GC methods.

1,000,000 Plates for Chiral Analysis Offers New Capabilities.

One of the more difficult analyses today is the separation of chiral (enantiomeric) compounds. Analysis of optical isomers are needed for drugs and or intermediates during chemical

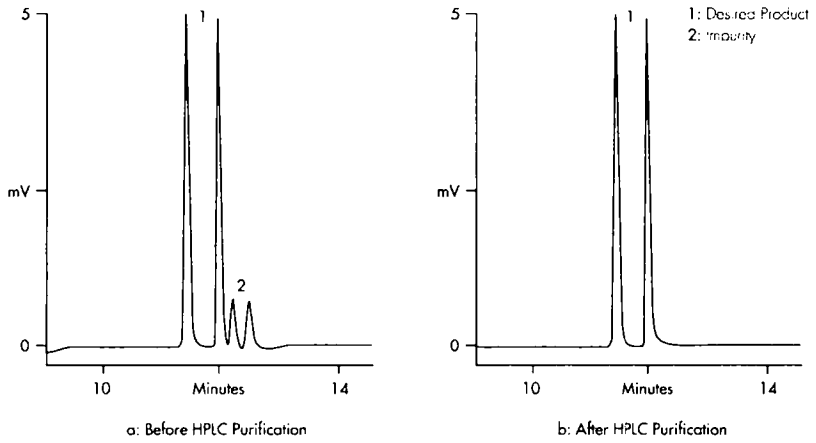
manufacturing, research or in pharmacokinetic studies. A variety of methods are available for the determination of chiral compounds including NMR, HPLC chiral specific columns, HPLC mobile phase modifiers, derivatization, or a combination of these methods. With the separation power of over 1,000,000 theoretical plates capillary electrophoresis separations offer new capabilities to perform chiral separations. In Figure 4a, a separation of a pharmaceutical product and its impurity, both of which were enantiomers, was developed on the Quanta 4000 using a 60 cm x 75 µm i.d. capillary. After purification by reverse phase HPLC, the purity of the chiral product was verified by capillary electrophoresis (Figure 4b). The CE chiral separation was achieved by adding ß-cyclodextrin as a chiral discriminator to the pH optimized electrolyte. Over 1,000,000 plates in this separation were generated with the total analysis requiring less than 13 minutes. The speed, selectivity and efficiency of this chiral separation rivals any analytical technique available today.

Summary.

Because CE is a relatively new technique without the extensive methods or data base of separations compared to established GC or HPLC methods, new methods and methodology are changing rapidly. At Waters, methods development techniques are constantly enhanced with new applications being generated daily in inorganics, foods and pharmaceutical analysis. Currently Waters Pharmaceutical Laboratory has developed methods for separation and quantitation using the Quanta 4000 capillary electrophoresis system for such compound classes as antibiotics, analgesics, vitamins, anesthetics, diuretics, surfactants and many drug intermediates. The very high separation efficiencies make it an ideal technique for solving difficult problems like chiral separations and for the determination of impurity profiles. The selectivity of CE complements those most commonly found in HPLC and as such provide the analyst with a powerful combination of techniques for the analysis of complex mixtures of molecules and the determination of absolute sample purity.

To learn more about fast capillary electrophoresis separations on Waters Quanta 4000 Capillary Electrophoresis System please check box 8 on the attached reply card. To learn more about computer data stations for capillary electrophoresis please check box 9 on the attached reply card. Waters periodically writes Applications Briefs and Prescriptions for Success to provide the most recent information available on new separations technologies. Check box 10 on the attached reply card for details.

Figure 4: Direct Chiral Analysis by CE of a Racemic Pharmaceutical Product and Its Impurity.



In this example, CE was used to verify peak purity after reverse phase HPLC purification.

Waters Expert Ease™ System Suitability Software.

by Mark Andrews, Waters Product Marketing Manager

Waters VAX Based 845/860 Chromatography Workstations Increase Laboratory Throughput.

Waters System Suitability Software increases laboratory throughput by summarizing the data and providing a quick visual confirmation of system performance. The suitability software saves valuable time by providing summary graphics and reports eliminating manual calculations such as plate count, resolution, tailing factors and relative standard deviation. More accurate results are obtained because the system suitability software directly and automatically accesses chromatographic data, eliminating the possibility of transcription errors.

System Suitability Parameters Provide Calculation Flexibility.

The system suitability software allows flexibility in the calculation of plate count and tailing factors. In addition to the USP tangent plate count method and the USP tailing factor method (5% above baseline), reports can be customized to include up to six other

methods of calculating these factors without custom programming. In addition to multiple calculations, the software includes an option to report values which exceed the minimum and maximum values entered for each system suitability parameter. Baseline noise and drift calculations are available automatically for individual reports. A maximum % RSD (relative standard deviation) value can also be included for each suitability parameter. Any calculated values outside the acceptable limits can be flagged as an outlier value on reports.

Customize Reports Without Writing Computer Programs.

The Suitability Custom Report facility allows you to tailor suitability reports to meet your laboratories needs. Without requiring computer programming, the system suitability report content and sequence can be customized. Choices for reported values include plate count, resolution, tailing factor, peak area, peak height, selectivity, various peak width values, as well as several LC result parameters. Two custom derive fields are available to allow further tailoring of results, or to calculate additional parameters.

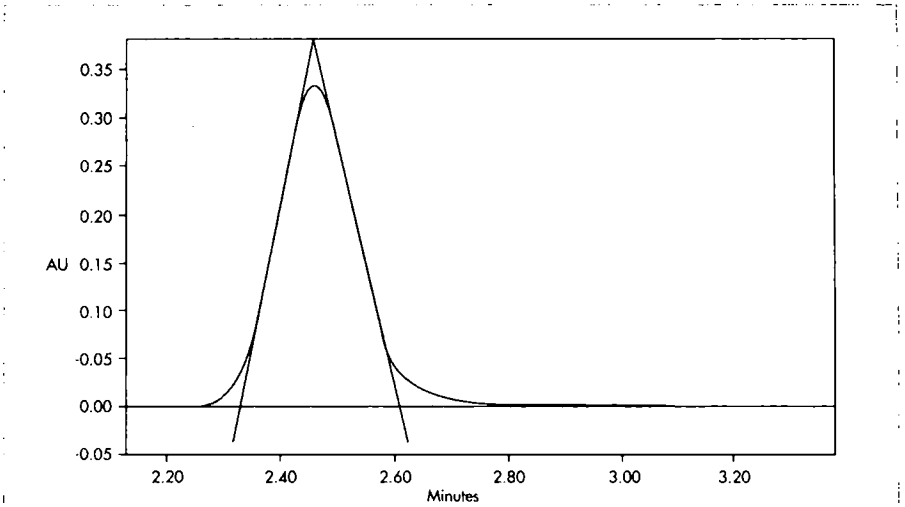
Automatic Visual Review of All Peak Calculations.

Waters Peak Plot is a tool which allows the chromatographer to visually review, for each peak, the actual widths and heights used in calculating plate count and peak tailing (Figure 1). To minimize transcription errors, parameters which are specified in the Custom Report Manager (e.g., Peak Name, Retention Time, etc.) are also automatically documented on the plot.

Increased Laboratory Throughput with Summary Statistics and Graphing.

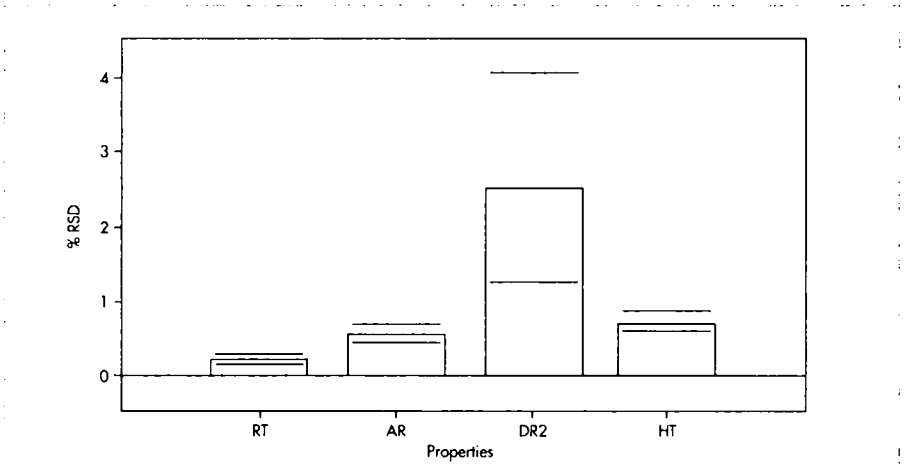
Waters System Suitability software increases your laboratory's throughput by summarizing the data and providing a quick visual confirmation of the system performance minimizing the need to look at individual data. An

Figure 1: Waters Peak Plot Shows Plate Count and Peak Tailing Calculations for Each Peak.



Peak plot provides the chemist a visual verification illustrating how the computer is calculating results for each peak.

Figure 2: Overall % RDS Generated by the System Suitability Software Package.



Overall % RSD bar graph reduces weeks of laboratory data to a concise, easy to interpret summary table. Individual peak parameters can also be monitored with this software package.

example of an overall method % RSD bar plot generated by the system suitability software package is shown in Figure 2. From multiple chromatographic runs, this software determined the overall % RSD for each parameter requested. The lines shown above and within the bars are the minimum and maximum % RSD for individual peaks in the calibration table. The bar graph is the mean % RSD for all the chromatographic runs. If the results on this summary plot are acceptable, then the individual data does not need reviewing. In many GMP/GLP protocols system suitability parameter trends are important. The system suitability trend plots include minimum and maximum acceptable value for each parameter specified. Plotting system suitability trends allows for quick visual inspection of data. Providing a quick visual inspection of data helps determine trends which may affect the reproducibility of the system or the overall % RSD of the method minimizing trouble shooting and maximizing uptime.

For more information on Waters System Suitability Software and how it can help maintain a GMP/GLP facility, check box 11 on the attached reply card.

Analysis of Inorganic Ions in a Vitamin Tablet Formulation.

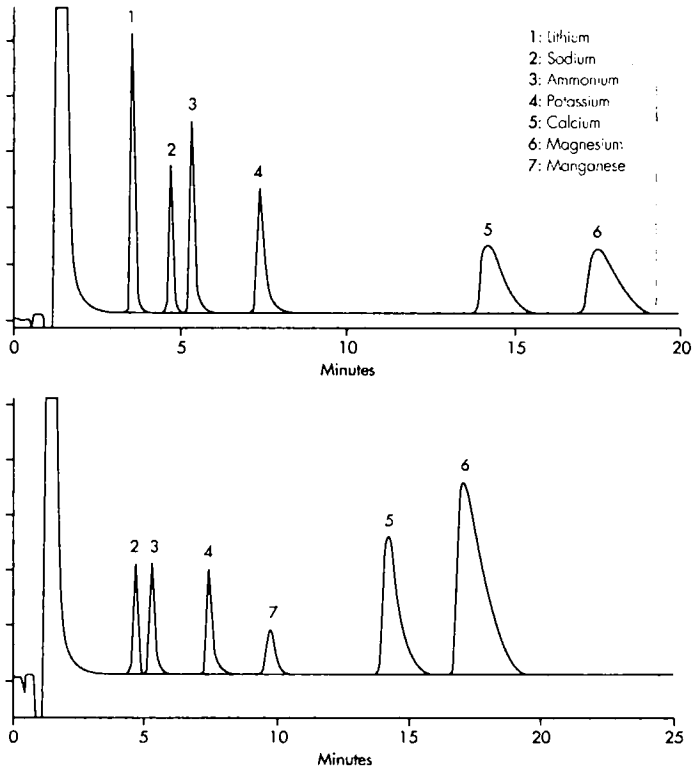
by Jim Krol, Waters Methods Development Chemist, Peter Jackson, Waters Applications Chemist and Allan Heckenberg, Laboratory Manager

Waters ActION™ Analyzer for Analysis of Alkaline Earths and Transition Metals In a Single Run.

Ion Chromatography (IC) is an analytical technique that is analogous to conventional HPLC analysis in many aspects. Sample introduction, pumping systems and data analysis are essentially identical to HPLC systems, however, the mobile phases, separation columns and detection systems are optimized for the separation and detection of a variety of ions.

In the analysis of a vitamin tablet, two major groups of ions are analyzed, the alkaline earths, and the transition metals. The analysis of the alkaline earth cations; sodium, potassium, calcium,and magnesium using conventional sulfonated cation exchangers, has until recently, required the use of two separate sets of chromatographic conditions, one for monovalent cations, another for divalent cations. In some cases gradient separations have been used to provide a single analysis, however, overall run times, cost per analysis and detection limits have been little improved over performing two isocratic separations.

Figure 1: Cation Analysis of Standards and Vitamin Tablets.



Cations are separated on a Waters IC Pak M/DC using a PDCA/citric acid eluent and Waters 431 Conductivity Detector.

Simplify the Analysis of Mono and Divalent Cations on Waters Unique Coordination Chemistry Columns.

The introduction of *coordination chemistry* columns, using recently developed functional groups, permit two simultaneous retention mechanisms to occur, giving a separation of mono and divalent cations in one isocratic chromatographic run. Monovalent cations are retained and separated through an essentially conventional cation exchange mechanism using carboxylic acid functional groups, the divalent cations are retained and separated through coordination with malonic acid functional groups. The eluents used with this separation mechanism are compatible with high sensitivity conductivity detection, resulting in a single analysis method that is simple, less costly, more rapid and sensitive than separate isocratic methods.

Waters Dynamically Coated Columns Provide Fast Analysis of Transition Metals.

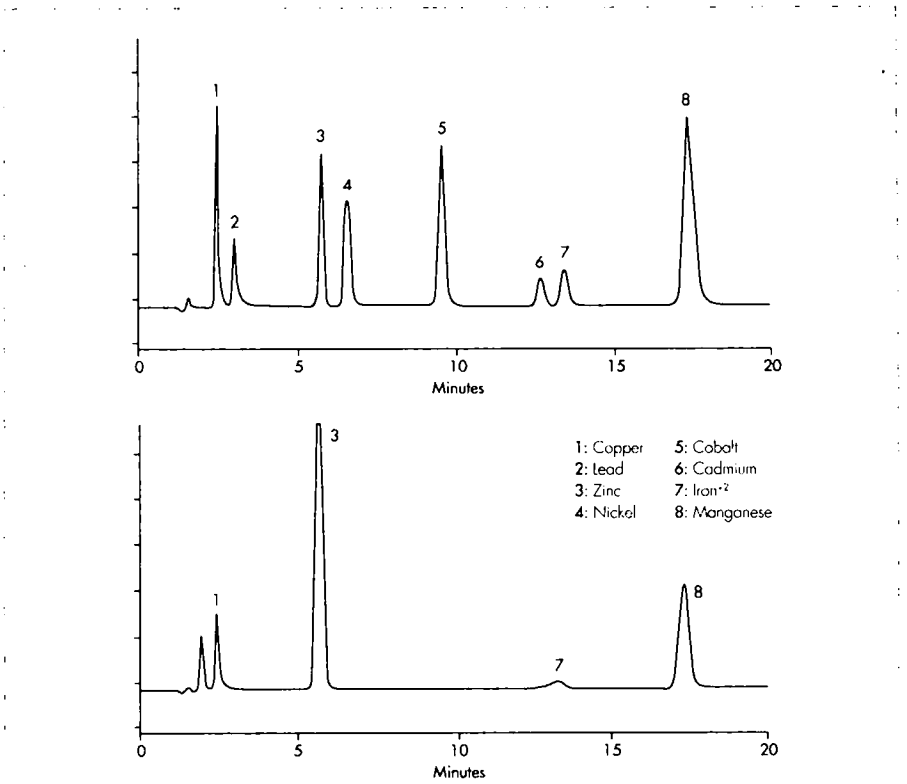
Transition metals are separated using the *dynamic coating* process . A reverse phase C_{18} column is operated with an eluent containing sodium octane sulfonic acid (NaOS). The NaOS component of the eluent forms a constantly renewed cation exchange surface on the packing material. This process allows for extremely high efficiency separations, with exceptional retention time stability, even over wide ranges of analyte concentration. The metals are eluted from the column by chelation with the tartaric acid component of the eluent. Selective and sensitive detection for the transition metals is achieved by the addition of a post column reaction solution containing Pyridyl-azo-resorcinol (PAR). The resulting metal-PAR complex absorbs strongly at 500 nm, resulting in excellent detection limits for transition metals. Detection specificity and selectivity is excellent with minimal interference from matrix organics and anions, or other cations commonly found at relatively high levels in pharmaceuticals such as sodium, calcium and magnesium.

WISP Autosampler Provides High Volume Throughput.

Excellent quantitative results with precision and accuracy typically better than 3% are routinely achieved for vitamin tablet matrices for both the cation and transition metal analyses. Both the cation method with coordination group chemistry separations, or the transition metals separated by dynamically coated C_{18} columns provide method detection limits in the low ppb range. In common with HPLC analysis, ion chromatography systems may be automated for routine or high volume sample throughput, with the addition of a WISP sample processor.

The Ion chromatograph used consisted of a Waters ActION Analyzer, with a Waters 431 Conductivity Detector for cation analysis and a Waters Reagent Delivery Module with a Waters 490 UV/Visible detector used for transition metal analysis. Columns and eluents are as noted in Figures 1 and 2.

Figure 2: Transition Metal Analysis on Waters *Dynamically* Coated Columns.



Transition metals are separated on a Waters Dynamically Coated column with a NaOS/Tartrate/ Acetonitrile eluent using post column detection with a PAR and a Waters 490 UV/Vis Detector.

Summary.

The analysis of commercial vitamin tablets by ion chromatography is a simple and effective process. Automated systems permit the analysis of around thirty samples in a eight hour day, for up to fourteen different cations. The IC methods detection limits, precision and accuracy, compare favorably with alternate analytical methodologies. Since IC employs both a separation and detection step, matrix interference commonly encountered in spectroscopic techniques are minimized. In addition to offering comparable detection sensitivity, the ability to simultaneously analyze multiple cations is easily achieved from one sample injection. The ease of use, effective automation and availability of high quality data processing software make ion chromatography the choice for today's vitamin analysis.

References:

1. Waters Ion Chromatography Cookbook, Method #M-301

For additional information on instrumentation for the analysis of alkaline earths and transition metals please check box 12 on the attached reply card.

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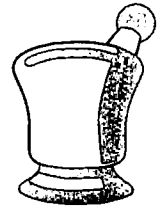

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