Analysis of human rhinovirus (common cold virus) in viral preparations by CZE

Application Note

Proteomics

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Abstract

Determining the purity of viral preparations obtained from tissue culture is a common task in virological laboratories. Usually, a sample is heat-denatured in the presence ofsodium dodecyl sulfate (SDS) and the viral proteins are analyzed by polyacrylamide gel electrophoresis (SDS-PAGE). However, this method gives no indication as to whether the virus was initially intact. Since viruses, like other colloidal particles, have a charged surface, differential migration in an electric field enables their separation and characterization. Capillary zone electrophoresis (CZE) is thus ideally suited to analyze native viruses as it combines a short analysis time with small sample consumption[1,2]. In addition, CZE enables the investigation of virus-ligand interactions in free solution.

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Experimental

Rapid analysis of human rhinovirus (HRV) preparations was carried out in an untreated fused silica capillary using sodium borate buffer at pH 8.3 as the background electrolyte (BGE). (SDS) was added to the BGE at a concentration of 10 mmol/L to prevent wall adsorption and aggregation of the virions. The samples were obtained from virus, propagated in HeLa cells grown in suspension cultures, by differential ultracentrifugation and zonal sucrose gradient sedimentation according to established protocols.

Results

Figure 1 shows the electropherogram of a typical viral preparation with one main peak, migrating at 4.5 minutes. The UV spectrum of this peak exhibits a maximum at 260 nm, indicating the presence of nucleic acids. The identity of the main peak as native virus was confirmed by a number of indirect methods – heat denaturation of the virus prior to CZE and enzymatic treatment of the degradation products immunodepletion with monoclonal antibodies or infectivity assays of the fractions collected post-column. The results allow the unambiguous identification of the main peak as native HRV. The minor peaks in the electropherograms are contaminants present at various amounts in the different viral preparations. The percentage of pure virus present in the sample can be determined by calculating the area of the virus peak as a percentage of the total peak area. This can be performed either at 205 nm (reflecting the absorption of all preparation components), or at 260 nm, where only the absorption derived from nucleic acids is measured..

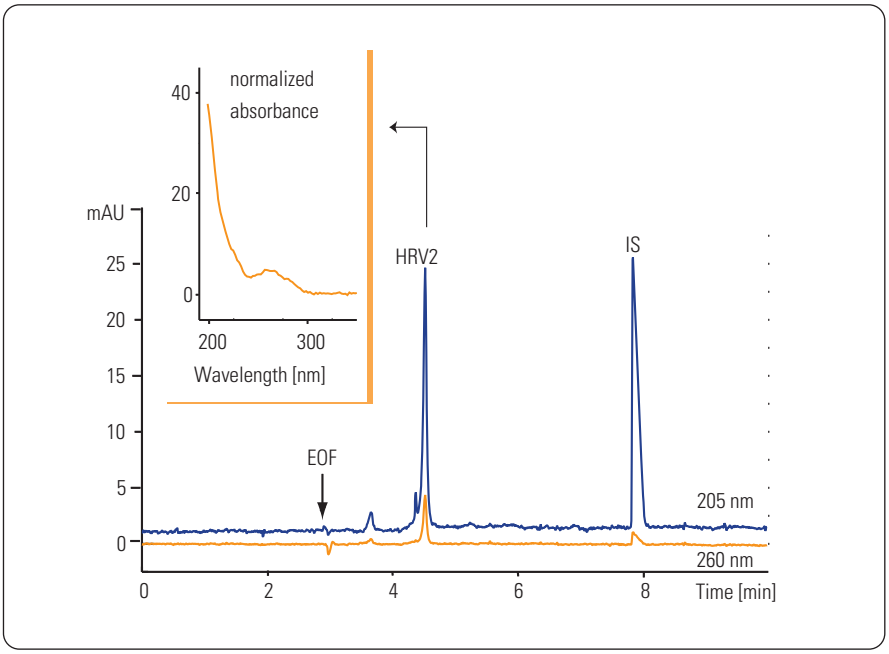

Figure 1
Electropherogram obtained from a typical viral preparation. UV-spectrum of the HRV 2 peak.

Chromatographic conditions

Sample:	HRV serotype 2 (HRV2), 0.25 mg virus per mL in 1:10 BGE without SDS. Internal standard (IS) o-phthalic acid
Injection:	50 mbar, 9 sec
Capillary:	effective length 41.5 cm, total length 50 cm; 50 µm id
BGE:	100 mmol/L Na borate/boric acid, pH 8.3, 10 mmol/L SDS
Voltage:	25 kV, positive polarity
Temperature:	20 °C
Detection:	diode array detector, 205 and 260 nm
Preconditioning:	between runs, flushing with 0.1 mol/L NaOH, water, BGE, 2 min each at 1 bar

References

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