SIMULTANEOUS QUANTITATIVE ANALYSIS OF MULTIPLE ATYPICAL ANTIPSYCHOTIC DRUGS IN HUMAN PLASMA BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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AIM

Development of a rapid, robust and sensitive method for the therapeutic monitoring of antipsychotic drugs widely used in the UK for the treatment of schizophrenia and schizo-affective disorders.

INTRODUCTION

- Schizophrenia is a serious illness that affects about 1 in every 100 people at some time in their life[1]
- Estimated cost of treatment is 2.5 % of healthcare costs in developed countries (for the NHS in England 2004-5 = ca. £2 billion)[2]
- Atypical (or second generation) antipsychotics have an effect on the negative and positive symptoms

Why is TDM of antipsychotics required?

- Compliance (adherence, concordance): poor or partial compliance with antipsychotic medication is common possibly due to adverse reactions
- Optimisation of therapeutic dosage
  - Is dosage adequate?
  - Is the patient at risk from dose-related toxicity (adverse drug reactions)?
- Investigation of drug interactions/metabolic influences (renal failure, liver disease)

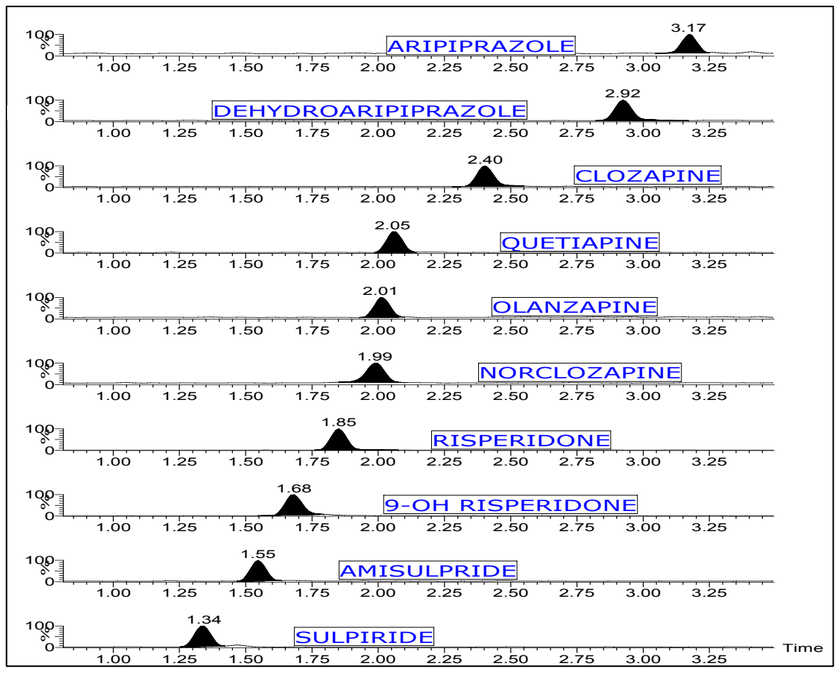

Figure 1. Extracted human plasma calibrator containing all compounds at a concentration of 1µg/L

METHODS

SAMPLE PREPARATION

Protein precipitation using methanol containing internal standard Imipramine-D3.

CHROMATOGRAPHY

ACQUITY UPLC system

Column: Waters XTerra MS C18 (2.1 x 50mm, 2.5µm), 30°C
Mobile phase: A=5mM Ammonium acetate, pH 10
B=Acetonitrile
Gradient: 20% B up to 80% B over 4 minutes (5.5 minute cycle time)

MASS SPECTROMETRY

Waters Quattro Premier XE mass spectrometer used in electrospray positive ionisation mode with a collision cell pressure of 3.5 x 10^{-3} mbar (argon)

Compound	Precursor ion (*m/z*)	Product ion (*m/z*)	CV (V)	CE (eV)
Amisulpride	370	242	40	30
Aripiprazole	448	176	50	30
Dehydroaripiprazole	446	285	40	25
Clozapine	327	270	45	20
Norclozapine	313	192	45	40
Olanzapine	313	256	40	25
Risperidone	411	191	45	30
9-OH Risperidone	427	207	45	25
Sulpiride	342	112	45	25
Quetiapine	384	253	40	25

RESULTS

For all compounds, responses were linear over the investigated range (1–200 µg/L). Intra-assay precision and accuracy were acceptable with CV's for spiked QC samples < 11% and > 86%, respectively. The use of a simple protein precipitation was demonstrated to be very efficient and gave reproducible extraction recoveries > 75% for all analytes. The matrix effects were assessed and found to be acceptable with the sulpiride response being most affected (-31%). All compounds gave satisfactory stability in prepared samples over 24 hours (except for olanzapine that showed a loss of 33% over the assessed time period). The method was applied to the analysis of spiked calibration standards and authentic clinical samples (*n* = 100) which were also analysed externally against an established HPLC/UV method[3]. EQA samples (*n* = 6) were also assessed and all gave a deviation of < 22% from the method mean.

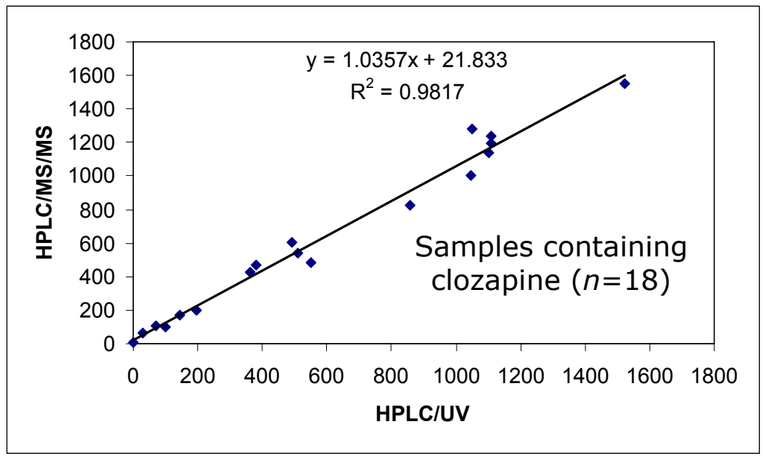

Figure 2. Comparison of patient samples analysed using an established HPLC/UV method[3] against the developed HPLC/MS/MS method

CONCLUSIONS

- Rapid and sensitive solution for the high-throughput monitoring of schizophrenia and schizo-affective disorder patients
- Simple protein precipitation sample preparation step
- Method validation demonstrates robustness with adequate linearity for all compounds over the investigated range
- Developed method shows good comparison to an established HPLC/UV method[3] with an 3 fold decrease in runtime and no liquid/liquid extraction needed
- Approximate LOD's range from 0.5 – 0.05 µg/L

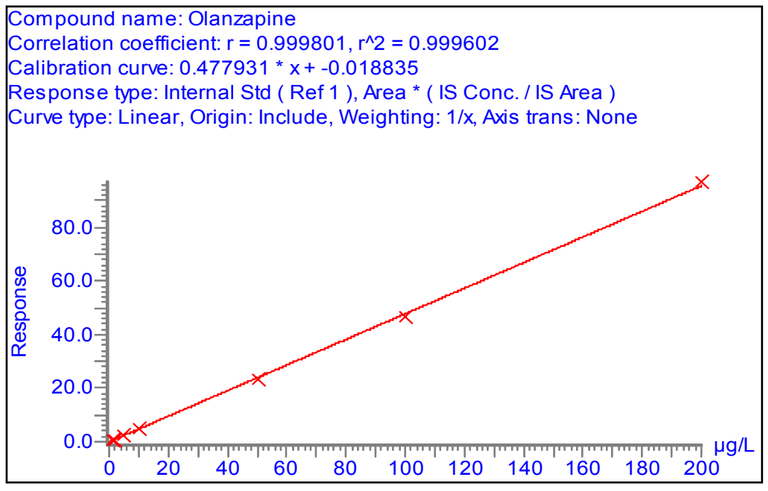

Figure 3. Typical response for extracted human plasma calibrators containing olanzapine

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1. R. Mangalore, M, Knapp. Cost of schizophrenia in England. J. Ment. Health. Policy. Econ. 2007; 10: 23-41
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3. P.T. McCarthy, S. Hughes, C. Paton. Measurement of clozapine and norclozapine by high performance liquid chromatography with ultraviolet detection. Biomedical chromatography 1995; 9: 36-41

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