Micro-extraction techniques in analytical toxicology

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Introduction

Simple micro-extraction procedures have been used in our laboratory for the analysis of lipophilic drugs and metabolites in plasma for many years [1]. A large excess of solvent is often unnecessary. Butyl acetate [2] and methyl *tert*-butyl ether (MTBE) [3] give efficient extraction of many drugs and metabolites from plasma at an appropriate pH, and form the upper layer thus simplifying extract removal. These solvents are cheap, available with high purity, and do not interfere in NPD, ECD, or MS. HPLC eluents that use a high proportion of an organic component [4] also allow MTBE extracts to be analysed directly (MTBE has a relatively low UV cutoff, 215 nm). Unlike some other ethers, MTBE does not form peroxides at ambient temperature and thus stabilisers are unnecessary.

A well defined LLE system is robust and cost-effective. Appropriate repeating dispensers are used for solvent and reagent additions. Use of clear glass test-tubes (60 x 5 mm i.d., Dreyer tubes) rather than plastic tubes as extraction vessels simplifies extract removal via a syringe or fine-tipped plastic Pasteur pipette and minimises the risk of contamination with the aqueous phase. Of the mixing methods available, vortex-mixing is the quickest and the most efficient method for relatively small volumes (50-200 µL) and is safe if performed in a fume hood or safety cabinet. Use of an appropriate centrifuge with sealed rotors can give efficient phase separation whilst minimizing (i) emulsion formation, (ii) tube breakage, and (iii) the risks associated with centrifugation of solvents and of potentially infective specimens.

Applications

This micro-extraction approach is simple, inexpensive, suited to batch processing and to emergency work, and has been used for hundreds of thousands of analyses since it was introduced in our laboratory [Fig. 1]. However, it is not amenable to automation and hence is labour intensive if a large number of samples are to be assayed, although a trained operator can process 100 tubes in ca. 2.5 h. The sample injection step, however, can be automated. With conventional HPLC (5 µm aps packings, <14 min analysis time) the chromatographic system defines the sample capacity [4]. Inter-assay RSDs (lowest IQC specimens) of <15 % over 6 months are typical for most analytes when assayed by HPLC [Fig. 2; Table 1].

Fig. 1. Outline of simple GC extraction procedure

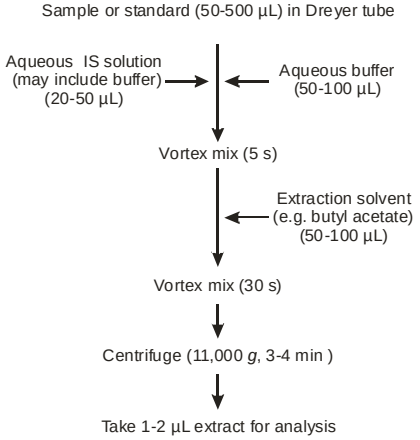


Fig. 2. Outline of simple HPLC extraction procedure

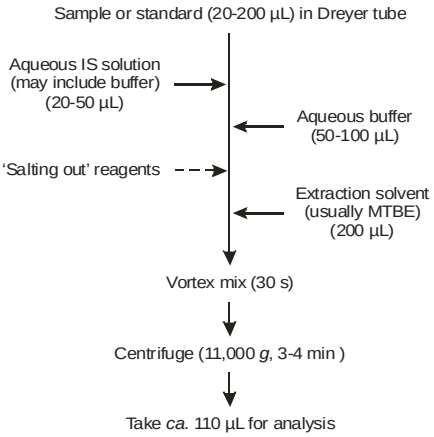
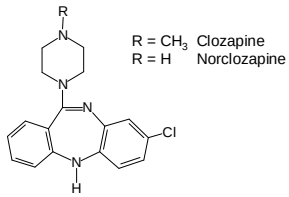


Table 1. Summary of some liquid-liquid micro-extraction drug and metabolite HPLC analyses routinely offered at the Medical Toxicology Unit

Drug	*Metabolite*	*Sample (µL)*	*Extract pH*	*LLoQ (µg/L)*
Amiodarone	**Noramiodarone**	**100**	**4.6**	**100**
Bupivacaine		**100**	**14**	**100**
Chloroquine		**200**	**14**	**10**
Citalopram	**Norcitalopram**	**200**	**10.6**	**5**
Clozapine	**Norclozapine**	**200**	**10.6**	**10**
Disopyramide		**200**	**14**	**100**
Doxazosin		**200**	**14**	**10**
Flecainide		**50**	**9.2**	**5**
Hydroxy-chloroquine		**200**	**14**	**10**
Lidocaine		**100**	**14**	**100**
Metoprolol		**100**	**14**	**10**
Mexiletine		**200**	**9.2**	**10**
Mirtazapine	**Normirtazapine**	**200**	**10.6**	**1**
Paroxetine		**200**	**10.6**	**1**
Procainamide	**Acecainide**	**200**	**14**	**100**
Propafenone	**5-Hydroxy-propafenone**	**200**	**9.2**	**50**
Propranolol		**50**	**14**	**2**
Quetiapine		**200**	**9.2**	**2**
Quinidine		**100**	**10.6**	**100**
Quinine		**100**	**10.6**	**100**
Sertraline	**Norsertraline**	**200**	**10.6**	**5**
Trazodone		**100**	**10.6**	**50**
Venlafaxine	**O-Desmethyl-venlafaxine**	**200**	**10.6**	**5**
Verapamil	**Norverapamil**	**200**	**14**	**2**

HPLC of Clozapine

The HPLC of clozapine provides a typical example of the use of SCX-modified silica packings in the analysis of basic drugs via direct injection of MTBE extracts [4]. Clozapine is used to treat refractory schizophrenia. Dosage is some 300-900 mg/d. *N*-Desmethylclozapine (norclozapine) accumulates in plasma to concentrations similar to those of clozapine (typically 0.1-1.5 mg/L).



Clozapine and norclozapine are assayed in duplicate by HPLC of a solvent extract of plasma (Fig. 3). In summary, sample or standard (200 µL) are added to a 60 x 5 mm i.d. glass (Dreyer) test-tube. Aqueous nortriptyline (5 mg/L; 20 µL) & Tris buffer (2 mol/L, pH 10.6; 100 µL) are added and, after vortex-mixing (5 s), MTBE (200 µL) are added. After vortex-mixing (30 s) and centrifugation (9950 g, 4 min – Hettich EBA12), 120 µL of the extract (upper phase) are transferred to an autosampler vial. Assay calibration is by means of human serum standards (0.1, 0.2, 0.5, 0.8, 1.0, 1.5 mg/L both compounds). Internal QC samples (0.15, 0.4, 1.2 mg/L both compounds) are analysed between every 5 samples. External QA is Heathcontrol (monthly)

HPLC conditions - Clozapine assay

Column: 150 x 4.6 mm i.d. Waters Spherisorb S5SCX (5 µm aps); Eluent: 35 mmol/L ammonium perchlorate in methanol, apparent pH 6.7, filtered (Millipore 0.45 µm) before use; Detection: UV, 240 nm. Flow-rate: 1.0-1.5 mL/min; Temperature: ambient; Injection: 70-100 µL extract

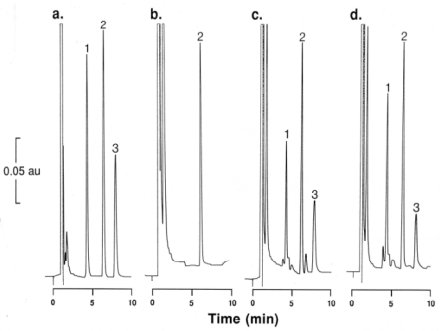


Fig. 3. Clozapine assay. Samples: a. Standard (0.5 mg/L both compounds); b. 'Blank' serum; c. Patient serum (dose 500 mg/d; clozapine & norclozapine 0.30 & 0.29 mg/L, respectively); d. Patient serum (dose 900 mg/d; clozapine & norclozapine 0.43 & 0.23 mg/L, respectively). Injections: 70 µL MTBE extracts. Peaks: 1 = clozapine, 2 = nortriptyline (internal standard), 3 = norclozapine

References

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3. Flanagan RJ, Storey GC, Holt DW. Rapid high-performance liquid chromatographic method for the measurement of amiodarone in blood plasma or serum at the concentrations attained during therapy. J Chromatogr 1980; 187: 391-8.

4. Flanagan RJ, Harvey EJ, Spencer EP. HPLC of basic drugs on microparticulate strong cation-exchange materials - a review. Forensic Sci Int 2001;121:97-102.