Journal of Chromatography, 225 (1981) 189—195
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 914

Note

Evaluation of C_{18} Sep-Pak cartridges for biological sample clean-up for tricyclic antidepressant assays

N. NARASIMHACHARI

Department of Psychiatry, Box 710, Medical College of Virginia, Richmond, VA 23298 (U.S.A.)

(First received November 21st, 1980; revised manuscript received April 1st, 1981)

The most commonly used method for the clean-up of biological samples for quantitation of tricyclic antidepressants is the extraction method. The various solvents used for extraction, extraction and derivatization procedures for gas chromatographic (GC) and GC—mass spectrometric (MS) methods have recently been reviewed [1]. In our studies we have used *n*-hexane—isopropanol (9:1) for extraction and a three-step extraction procedure for clean-up of biological samples, such as plasma and urine for tricyclic antidepressant assays [2]. More recently Waters Assoc. (Milford, MA, U.S.A.) have introduced a variety of small packed (Sep-Pak) cartridges for sample clean-up for analysis by liquid chromatography or GC. A few reports have recently appeared in the literature on the use of C_{18} Sep-Pak cartridges for the clean-up of serum or plasma samples for nucleoside and warfarin analysis [3, 4]. In view of our interest in plasma tricyclic antidepressant assays we have now investigated the use of C_{18} Sep-Pak cartridges for separating tricyclic antidepressants and their desmethyl metabolites from plasma and urine samples and evaluated the advantages, specifically sample cleanliness, specificity, efficiency, reproducibility, over the conventional extraction method.

EXPERIMENTAL

Materials

All solvents are pesticide grade (Mallinckrodt, St. Louis, MO, U.S.A.). Tricyclic antidepressant drugs were all obtained from pharmaceutical research laboratories as mentioned in a previous report [2]. d_4-Imipramine and d_4-desipramine were used as internal standards for all tricyclic drugs [2]. Stock solutions of hydrochlorides of internal standards were prepared separately

0378-4347/81/0000—0000/$02.50

in glass distilled water to contain 1 mg/ml of d_4-imipramine and d_4-desipramine as base. A mixture of 10 µl of each standard was then diluted to 1 ml with water to give a working standard containing 10 ng/nl of each of the internal standards. In addition dideuteromethyl amitriptyline and dideuteromethyl doxepin were prepared by reduction of N-formyl derivatives of nortriptyline and desmethyl doxepin using lithium aluminum deuteride [5, 6]. C_{18} Sep-Pak cartridges were purchased from Waters Assoc.

Working standards

Solutions containing 100—500 ng/ml of different tricyclic drugs in water, and drug-free plasma samples were prepared by adding standard solutions of the drug. The drug pairs, imipramine—desipramine, amitriptyline—nortriptyline, doxepin—desmethyldoxepin and protriptyline were added to separate tubes containing 2 ml of water or control plasma samples. Fifty microliters of internal standard solution (500 ng each of d_4-imipramine and d_4-desipramine) were added to each one of the tubes. A mixture of all the seven drugs (200 ng each) and two internal standards (500 ng) was added to a control plasma sample. In another set d_2-amitriptyline and d_2-doxepin were used as internal standards for amitriptyline and doxepin, respectively.

Carbonate—bicarbonate buffer

A mixture of 5 g each of sodium carbonate and sodium bicarbonate was dissolved in 100 ml of water (pH 9.8) and stored in a refrigerator.

C_{18} Sep-Pak clean-up procedure

The cartridge is activated by passing 2 ml of methanol by pressurizing through a plastic or glass syringe followed by 2 ml of distilled water.

Sodium carbonate—bicarbonate buffer (0.5 ml) was added to the aqueous solution, or plasma solution, the mixture thoroughly mixed on a vortex mixer, and passed through the cartridge via the syringe at a flow-rate not greater than 5 ml/min followed by 1 ml of washings from the sample tube. The cartridge was then washed by passing 2 × 2 ml of distilled water through it. The effluent of the sample and washings were collected, and saved for determination of unabsorbed drugs.

Ten milliliters of solvent mixture, hexane—isopropanol (9:1) were passed through the cartridge and the eluate collected in a 15-ml glass stoppered centrifuge tube. The eluate consisted of 0.4 ml of aqueous layer from the void volume of the cartridge, and was drawn off and discarded. The organic layer was evaporated under a current of nitrogen, the residue derivatized to the trifluoroacetyl (TFA) derivative using N-methyltrifluoroacetamide according to the method described in an earlier report [2].

Gas chromatography—mass spectrometry

The GC and GC—MS—selected ion monitoring (SIM) conditions for quantitation of all tricyclic antidepressant drugs have been previously described in detail [2]. The same conditions were used in this study. All quantitations were carried out using the electron impact mode with ionization potential 70 eV, source temperature 260°C, separator temperature 250°C. Therefore

for amitriptyline and doxepin, the corresponding N-dideuteromethyl derivatives were used as internal standards and ions *m/z* 58 and 60 were monitored for SIM. In all cases in these initial evaluation studies, the complete mass spectra of the drug peaks were obtained from plasma and urine samples in order to ascertain the sample cleanliness.

RESULTS

Column efficiency

The spent efficient including water wash was extracted at pH 10.0 into hexane—isopropanol (9:1) and worked up according to the procedure described earlier [2]. Analysis by GC—MS—SIM did not show any trace of the drugs indicating complete absorption of the compounds by the C_{18} Sep-Pak column. Similarly in preliminary experiments we collected the first 10 ml of solvent eluent and separately 5 ml of second eluent. The second fraction was analyzed separately for the drug quantitation. In most cases (80% of the samples), the drug content was undetectable and in 20% of the samples it was less than 5%. It can be concluded that the extraction efficiency is over 95%. Further, recovery studies with known concentrations (100—200 ng/ml) of added standards to drug free plasma showed a mean recovery of 93.8 ± 3.5% (n = 4).

The calibration curves were linear and similar to those reported for the extraction method [2].

Final sample cleanliness

In one experiment a mixture of seven drugs and internal standards was processed through the C_{18} Sep-Pak cartridge and the drugs were simultaneous-

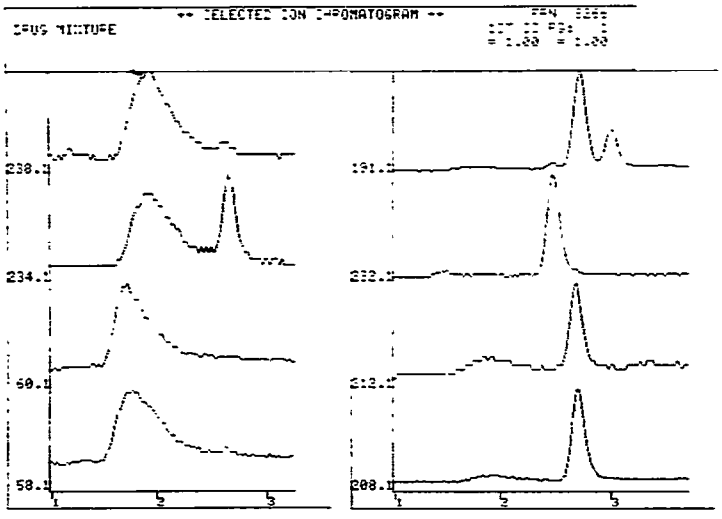

Fig. 1. SIM recordings of drug mixture and internal standards. Peaks: *m/z* 58, imipramine, doxepin and amitriptyline; *m/z* 60, d_2-doxepin and d_2-amitriptyline; *m/z* 191, protriptyline TFA; *m/z* 208, desipramine TFA; *m/z* 212, d_4-desipramine TFA; *m/z* 232, nortriptyline TFA; *m/z* 234, imipramine, desmethyl doxepin TFA; *m/z* 238, d_4-imipramine.

ly analyzed by GC—MS—SIM after derivatization. The selected ion recordings are shown in Fig. 1.

As illustrative examples, a few of the mass spectra obtained from patient plasma and urine samples processed through the Sep-Pak cartridges are given in Figs. 2—5. The mass spectra were identical with those of reference spectra obtained using five compounds under the same GC—MS conditions. In over 20 samples that we have so far processed by this method under the GC—MS conditions we have used, the spectra indicated that the sample peaks were clean and contained only the drug or the drug and internal standard. The samples were at least as clean as those obtained by the conventional extraction

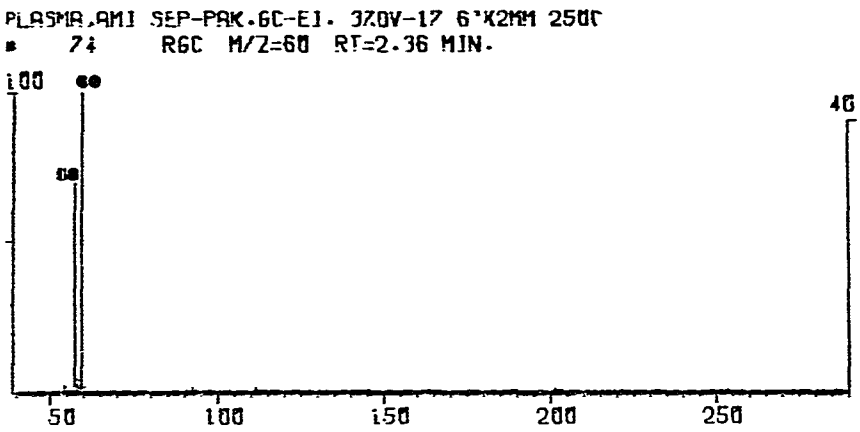

Fig. 2. Mass spectrum of amitriptyline peak from patient plasma sample processed by C_{18} Sep-Pak cartridge. Peaks: *m/z* 58, amitriptyline; *m/z* 60 and 281, d_3-amitriptyline.

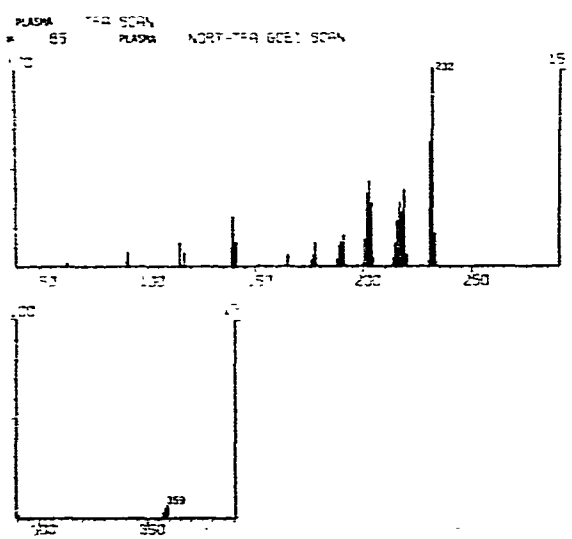

Fig. 3. Mass spectrum of nortriptyline TFA from patient plasma sample, processed by C_{18} Sep-Pak cartridge. Peaks: *m/z* 232, base peak; *m/z* 359, molecular ion.

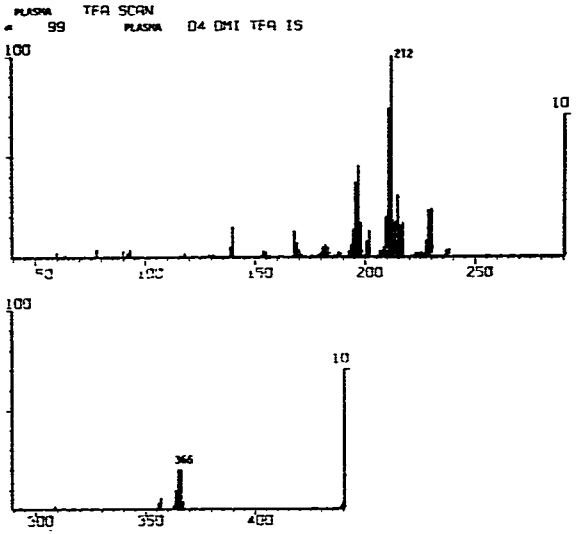

Fig. 4. Mass spectrum of d_4-desipramine TFA from the plasma sample. Peaks: *m/z* 212, base peak; *m/z* 366, molecular ion.

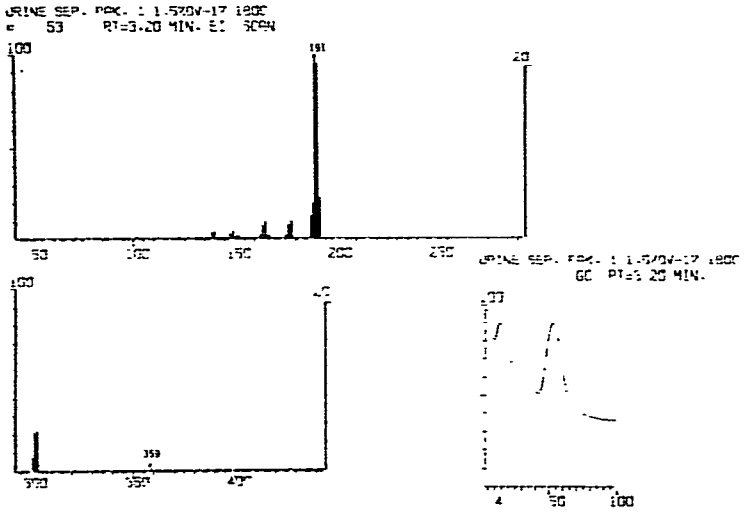

Fig. 5. Mass spectrum of protriptyline TFA from a patient urine sample.

method we have been using in our laboratories. The SIM recording for protriptyline assay is shown in Fig. 6.

Quantitative reproducibility and sensitivity

Parallel determinations of clinical samples (n = 11) were carried out for all tricyclic drugs by the two methods, one using the Sep-Pak and the second by extraction method. The results are presented in Table I. One interesting observation made during this study was that when equal aliquots of the final derivatized product of samples processed by Sep-Pak or extraction method

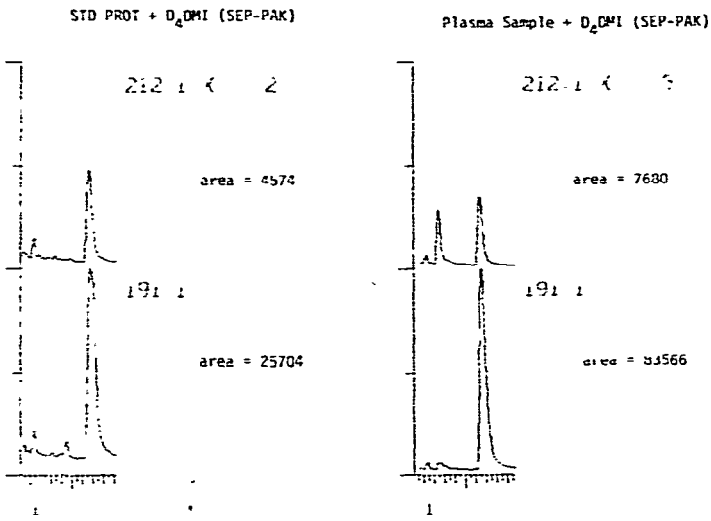

Fig. 6. SIM recording of *m/z* 191 and 212 for quantitation of protriptyline and d_4-desipramine in patient plasma, processed by Sep-Pak method.

TABLE I

PARALLEL DETERMINATIONS FOR THE TRICYCLIC DRUGS BY THE SEP-PAK AND EXTRACTION METHODS

Drug	Sample	Sep-Pak (ng/ml)	Extraction (ng/ml)
Protriptyline	Plasma	218	212
	Plasma	198	202
	Urine	2740	2700
Amitriptyline	Plasma	164	160
Nortriptyline	Plasma	172	170
Imipramine	Plasma	135	129
Desipramine	Plasma	168	170
	Plasma	404	415
	Urine	489	502
Doxepin	Plasma	100	97
Desmethyl doxepin	Plasma	65	67

TABLE II

COMPARISON OF SIM PEAK AREAS OF *m/z* 212 FOR THE INTERNAL STANDARD, d_4-DESIPRAMINE BETWEEN EXTRACTION AND SEP-PAK METHODS

Sep-Pak	*n*	Extraction (Mean ± S.D.)	*n*
82,550 ± 4200	6	78,951 ± 8601	10
81,200 ± 4096	6	59,209 ±17,704	10

were injected into the GC—MS system, the peak areas from Sep-Pak samples were consistently higher than the peak areas from extraction samples (Table II). Further peak areas of internal standards added to biological samples varied more widely in the extraction method than in the Sep-Pak method (Table II).

DISCUSSION

In this preliminary study we have demonstrated the usefulness of C_{18} Sep-Pak cartridges for the clean-up of clinical plasma and urine samples for the analysis of tricyclic antidepressant drugs. We have also shown that the recovery of the drugs is almost quantitative (> 95%). The samples we analyzed by this method ranged in concentration from 8—410 ng/ml. In our routine clinical analysis for tricyclic antidepressants during the past two years by the extraction method, we have observed wide variations in extraction efficiencies between different plasma samples. The use of internal standards obviates this extraction problem but low recoveries can cause problems of sensitivity for quantitation of low levels of the drug. Our findings with Sep-Pak suggest more consistent and higher recoveries from plasma and urine samples. In one clinical sample where protriptyline was discontinued and desipramine was started, both drugs were monitored in the plasma sample in a single injection using a single internal standard (d_4-desipramine). The saving in time for the analysis using Sep-Pak cartridges is the most important favourable factor for this method. The three-step extraction method involves shaking, centrifuging, withdrawal of solvent or aqueous layers, in all the three steps. In parallel experiments we found that eight samples and two standards could be readied for GC—MS analysis in 40 min, while the conventional extraction methods took 150 min. Another advantage in the Sep-Pak cartridge is that the organic layer is quantitatively recoverable, while in the extraction method, only a fraction is recoverable, and sometimes losses are high due to emulsification. We are evaluating the Sep-Pak cartridges with a large number of clinical samples and if our preliminary findings are confirmed the Sep-Pak cartridges can replace the extraction method.

REFERENCES

1 Bruce A. Scoggins, K.P. Maguire, T.R. Norman and G.D. Burrows, Clin. Chem., 26 (1980) 5.
2 N. Narasimhachari and R.O. Friedel, Res. Comm. Psych. Psych. Beh., 4 (1979) 477.
3 R.A. Hartwick, D. van Haverbeke, M. McKeag and P.R. Brown, J. Liquid Chromatogr., 2 (1979) 725.
4 M.J. Fasco, M.J. Cashin and L.S. Kaminsky, J. Liquid Chromatogr., 2 (1979) 565.
5 N. Narasimhachari and R.O. Friedel, Anal. Lett., 13 (1980) 517.
6 J.-P. Thenot, T.I. Rup and O.J. Bouwsma, Anal. Lett., 13 (1980) 759.