Therapeutic Monitoring of Tricyclic Antidepressants in Plasma by Gas Chromatography

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We describe a comprehensive gas chromatographic analysis for therapeutic concentrations of amitriptyline, nortriptyline, imipramine, desipramine, doxepin, and desmethyldoxepin in plasma, with use of a nitrogen detector. All these drugs are extracted and chromatographed under identical conditions: Each tertiary amine tricyclic is well resolved from its secondary amine metabolite on a mixed-phase column and the concentrations of both are determined simultaneously, without derivatization. The lower limit of sensitivity is 10 μg/liter of plasma (2-ml sample). Analytical recoveries of the tertiary and secondary amines are 100 and 80%, respectively. Between-run CV’s for all of the drugs ranged between 5 and 7%.

Development of the alkali flame ionization detector, which is specific for compounds containing nitrogen and phosphorus, has made it feasible to monitor tricyclic antidepressants in plasma during therapy. Several methods in which this detector is used are available for measuring amitriptyline and nortriptyline (1–4) or imipramine and desipramine (5–7). However, the only published method for measuring all the antidepressants that one would expect to encounter in routine therapeutic drug monitoring involves the use of mass fragmentography to detect the drugs (8).

We have adapted the method used by Curry for chlorpromazine (9) so that it can be used to analyze for all the commonly prescribed antidepressants, the principal modification being the use of a nitrogen detector.

Materials and Methods

Chromatography

The gas chromatograph we used is a Model 990 instrument (Perkin-Elmer Corp., Norwalk, Conn. 06852) equipped with an alkali flame ionization detector, which is specific for compounds that contain nitrogen and phosphorus. The 1.8 m × 2 mm coiled glass column is packed with a mixed phase of 1% OV 17 plus 2% OV 225 by weight, coated from the same solution onto 80/100 mesh Chromosorb WHP (Applied Science Laboratories, State College, Pa. 16801). The chromatographic conditions are: column oven temperature, 215 °C; injector temperature, 260 °C; interface temperature, 270 °C. The carrier gas is helium, with a flow rate of about 20 ml/min. Hydrogen flow to the detector is about 1 ml/min; air flow is 85 ml/min. Detector current setting is 600–700.

Reagents

Ethanol/isopentyl alcohol, 98/2 by vol.
Toluene/isopentyl alcohol, 85/15 by vol.
Heptane/isopentyl alcohol, 97/3 by vol.

Stock solutions, 1.0 g/liter. Dissolve 25 mg of drug (as hydrochloride) in 25 ml of ethanol/isopentyl alcohol. To prepare 100 mg/liter dilute solutions, dilute the stock solution 10-fold with ethanol/isopentyl alcohol.

Calibration standards, 5 mg/liter. Dilute each of the dilute solutions of a tertiary amine and its corresponding secondary amine 20-fold with ethanol/isopentyl alcohol.

Solvants with internal standard, 50 μg/liter. Dilute 0.5 ml of the dilute solution of the appropriate internal standard to one liter with heptane/isopentyl alcohol.

Extraction Procedure

Pipet either 2 ml of patient’s plasma (samples) or 2 ml of drug-free plasma (standards) into Teflon-stoppered tubes containing 1 ml of saturated aqueous Na2HPO4. Add 0.1 ml of ethanol/isopentyl alcohol to the patient plasma. Add 0.1 ml of the appropriate calibration standard to the drug-free plasma (220 μg of free drug per milliliter). Add 10 ml of hexane/isopentyl alcohol containing the appropriate internal standard and extract by inverting the tubes by hand for 2 min. Centrifuge (3 min, 1000 × g) and transfer the organic (upper) layer to tubes containing 1 ml of dilute HCl (10 mmol/liter). Extract and centrifuge as above. Aspirate and discard the organic (upper) layer. Transfer most of the aqueous layer to conical tubes containing 1 ml of saturated aqueous Na2HPO4 and 0.1 ml of tolenue/isopentyl alcohol and extract for 15 s with a vortex-type mixer. Centrifuge (3 min, 1000 × g) and remove most of the aqueous (lower) layer. Centrifuge (2 min, 500 × g) to clearly separate the remaining layers and transfer the organic (upper) layer to micro-sample vials. Inject 5 μl into the chromatograph.

Results

Dilutions of 9, 44, 88, 176, 220, and 264 μg/liter of plasma (calculated as free base) were made for each drug. Relative peak heights and concentration were linearly related over this range of concentrations, which includes those ordinarily expected in the serum of patients who are receiving therapeutic doses of these drugs.

Amitriptyline and nortriptyline were measured simultaneously, with desipramine as the internal standard (Figure 1, left). The calibration curve for amitriptyline typically had a slope of 0.0054, an ordinate intercept of −0.04, and a correlation coefficient of 0.99. The curve for nortriptyline typically...
had a slope of 0.0040, an intercept of −0.02, and a correlation coefficient of 0.99.

Imipramine and desipramine were measured simultaneously with amitriptyline as the internal standard (Figure 1, middle). The calibration curve for imipramine typically had a slope of 0.0062, an ordinate intercept of −0.03, and a correlation coefficient of 0.99. The curve for desipramine typically had a slope of 0.0034, an intercept of −0.04, and a correlation coefficient of 0.98.

Doxepin and desmethyldoxepin were measured simultaneously with amitriptyline as the internal standard (Figure 1, right). The calibration curve for doxepin typically had a slope of 0.0034, an ordinate intercept of −0.02, and a correlation coefficient of 0.99. The curve for desmethyldoxepin typically had a slope of 0.0023, an intercept of −0.03, and a correlation coefficient of 0.98.

Within-run precision was determined by analysis of quadruplicate extractions of drug-free plasma that had been supplemented to a concentration of 133 ug/liter. Five sets of these analyses were run during two weeks. Table 1 shows the mean CV’s for the five sets. Between-run precision (Table 1) was determined by 20 analyses of the same plasma during a month.

Plasma from more than 500 patients’ samples was analyzed for these tricyclic drugs. Most of the patients were receiving therapeutic doses of other medications that often are administered concomitantly with tricyclics, such as benzotripine, clorazepate, diazepam, diphenhydramine, flurazepam, haloperidol, methyldopa, methylphenidate, perphenazine, trifluousazine, and trihexyphenidyl. We saw no peaks that interfered with analysis for the tricyclic antidepressants.

Discussion

The resolution between the various tertiary amine tricyclics and their secondary amine metabolites is not particularly good when OV 17 is used. A more polar liquid phase improves the separation between the tertiary amines and their demethylated metabolites (Table 2). However, as can be seen from the table, caffeine interferes with the analysis of amitriptyline on both OV 210 and OV 225. We avoided this interference, while preserving a better resolution between the tertiary-secondary amine pairs, by using a mixed-phase packing of 1% OV 17 plus 2% OV 225. Because OV 225 contains cyano-propyl groups, the bleed from this column produces relatively high background with a nitrogen detector. This packing should not be used for temperature programming without compensation for column bleed. We have found it to be suitable for use in isothermal analysis at 215 °C.

Of the phases we tried, only OV 210 separated nortriptyline and doxepin. Nortriptyline and imipramine are poorly resolved on all these columns and protriptyline may not be separated from desipramine on any of these phases. Several
authors have commented on the chromatographic interference between various tricyclics (2, 4, 5, 7, 8, 10). This interference should cause only minimal problems for therapeutic drug monitoring because different tricyclic antidepressants are very infrequently administered together in treating depression.

We conclude that our method presents an alternative to gas chromatography/mass spectrometry or to the use of a different procedure for the analysis of each of the antidepressant drug pairs. All the tertiary amine tricyclics are well resolved from their secondary amine metabolites and concentrations of each of the antidepressant drug pairs may be determined simultaneously, without derivatization. Interferences between different tricyclics preclude the use of this method for toxicological analysis.

Table 2. Relative (to Desipramine) Retention Times for Seven Drugs on Various Liquid Phases

<table>
<thead>
<tr>
<th>Compound</th>
<th>3% OV 17</th>
<th>3% OV 210</th>
<th>3% OV 225</th>
<th>1% OV 17 + 2% OV 225</th>
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<tbody>
<tr>
<td>ATb</td>
<td>.81</td>
<td>.72</td>
<td>.65</td>
<td>.71</td>
</tr>
<tr>
<td>NT</td>
<td>.91</td>
<td>.85</td>
<td>.84</td>
<td>.86</td>
</tr>
<tr>
<td>IMP</td>
<td>.88</td>
<td>.83</td>
<td>.76</td>
<td>.81</td>
</tr>
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<td>DMI</td>
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<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>DX</td>
<td>.92</td>
<td>.95</td>
<td>.86</td>
<td>.87</td>
</tr>
<tr>
<td>DDX</td>
<td>1.03</td>
<td>1.10</td>
<td>1.11</td>
<td>1.08</td>
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<tr>
<td>Caffeine</td>
<td>.49</td>
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<td>.67</td>
<td>.56</td>
</tr>
</tbody>
</table>

* All phases were coated on 80/100 mesh Chromosorb WHP.

b Abbreviations as in legend to Figure 1.

References