Measurement of Antidepressants by Liquid Chromatography: a Review of Current Methodology

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Antidepressant measurement by liquid chromatography (LC) has enhanced the therapy of patients who are being treated with some of the first generation tricyclic antidepressants; the merits of routinely monitoring the other antidepressants await further study. Currently, the role of LC is changing from dominant to complementary as a result of the recent availability of monoclonal antibody immunoassays with increased specificity. For successful application of LC, considerations would include designing the sampling protocol and matching assay that together are uniquely suitable for a particular laboratory. The assay should be simple, the sample preparation manual (liquid–liquid, solid-phase extraction columns), semi-automated, or automated. Normal- or reversed-phase columns with functionalities such as C-18, CN, C-6, and phenyl are used. Other useful LC variables include particle size, capping, ion-pairing, and recycling. This survey of LC methods includes the first and second generations, and new antidepressants such as alprazolam, amoxapine, bupropion, maprotiline, trazodone, and selected metabolites. Potential chromatographic interference by (e.g.) benzodiazepines and neuroleptics is addressed, followed by proposed guidelines for their resolution. Future developments are discussed.

Since the advent of antidepressant monitoring more than 20 years ago, the methodology has undergone major advances (1–7). At present, monitoring of the first-generation tricyclic antidepressants has become routine in most laboratories (6–9), by either chromatographic or immunoassay techniques (6, 10–12). The second generation tri- and tetracyclic and atypical antidepressants may be readily quantified by chromatographic techniques (6). The merits of their routine monitoring await further study, but it is important to update the current liquid-chromatographic (LC) methodology in view of the recently introduced immunoassays in which monoclonal antibodies are used, and to plan for the future strategy accordingly.

The merits of LC relative to other techniques are well recognized. Recently, the number of publications dealing with LC analysis has greatly diminished, indicating either that this technique has become routine in most laboratories or that the advent of immunoassay has shifted its dominance, or both.

Scoggins et al. (1), and Gupta and Molar (2) independently reviewed, in 1980, the various methodologies used for measuring tricyclic antidepressants before 1979. Wong (6) surveyed the LC methodology used in the United States between 1979 and 1984 for measuring antidepressants and has updated the methodology, including immunoassay and interference, up to 1986 (7). Norman and Maguire (13) also updated their previous review on methodology up to 1985. More recently, Fazio et al. (5) reviewed assay of tricyclic antidepressants by liquid chromatography. As is evident from these reviews, the following topics are of current interest: sample preparation (manual, on- and off-line automated); simultaneous assays of multi-antidepressants and metabolites; alternative column packing such as phenyl; “newer” antidepressants [alprazolam, amoxapine and its 7- and 8-OH metabolites, maprotiline and N-desethyl maprotiline, and trazodone and its metabolite, 1-(m-chlorophenyl)piperazine]; and assay interference. Bupropion and nomifensine, introduced to but recently withdrawn from the market, also stimulated some interest in monitoring (14–16).

In this review, I attempt (a) to examine the merits of LC for antidepressant monitoring in relation to immunoassay; (b) to recommend strategy for designing an LC assay for antidepressants; (c) to address briefly the sampling considerations in relation to methodology; (d) to examine the LC variables; (e) to review those LC assays published after the reviews of Scoggins et al., with emphasis on antidepressants used in the United States; (f) to address the issues of interference, and to recommend certain guidelines based on my own experience; and (g) to project future developments.

I. Merits of Liquid Chromatography

For most routine TDM drug assays, immunoassay is the predominant methodology (17). However, liquid chromatography still is used in certain clinical laboratories, such as in the reference laboratory and for measurement of drugs not readily measured by immunoassay. Indeed, liquid chromatography has, until recently, been the predominant method used for antidepressant measurement. With the recent introduction of the enzyme multiplied immunoassay technique (EMIT) for quantification with use of monoclonal antibodies for enhanced specificity (10), the role and popularity of LC may be changed.

Liquid chromatography is cost-effective, owing to the low reagent cost for the extraction solvent and mobile phase, which account for up to 10% to 15% of analysis costs. In addition, simultaneous assays of multi-drugs and metabolites are possible. For monitoring the newer antidepressants for toxicological purposes, liquid chromatography is often the preferred and perhaps the only practical means of quantification. Because routine use of liquid chromatography always presents minor technical problems, experience and simple procedure enhance its application.

II. Sampling Considerations

Because clinical response is correlated with the steady-state "trough" concentrations, blood sampling should be scheduled every one to three weeks, after initiation or change of therapy, depending on the biological half-life of the first-generation tricyclic antidepressant, and every one
to two weeks for new antidepressants such as maproline and trazodone with short half-lives. Having chosen a blood-collection device, one should sample blood about 30 min before the next dose or 12 to 14 h after.

Adequate blood-collection devices are now available for use in TCA monitoring. Several reviews and studies address the various problems (19–21). Previously, a plasticizer, tris(butoxyethyl) phosphate, used in the formulation of rubber stoppers, was shown to alter binding of tricyclic antidepressants to α1-acid glycoprotein, causing drug redistribution into erythrocytes (22, 23). Thus, the apparent drug concentration in serum was made artificially lower than the actual concentration. According to the recent studies by Orsulak et al. (20) and Suckow (21), gel serum-separator tubes also result in lower apparent drug concentrations in serum.

Based on some of these published studies and reviews, and on our experience, evacuated tubes containing EDTA would be acceptable if the whole blood is completely mixed immediately after venipuncture. The resulting plasma may be used for LC assay. Alternatively, serum collected in various sorts of tubes is suitable (7, 10, 20, 24, 25), and indeed is preferred for immunoassay, owing to the lack of fibrous material, which may clog the pipettor (10). TCA's are stable in serum for as long as four weeks at 4 °C (20). TCA's in serum or plasma are stable for several days at room temperature, and for up to 120 days when the specimen is stored frozen. An exception is bupropion (26), which exhibits in vitro degradation with half-lives of 52.2 and 11.4 h at 22 and 37 °C, respectively.

In a recent review, Suckow (21) concluded that serum from a plain collection tube would yield a 15% lower value for desipramine and nortriptiline than would plasma from an oxalated tube, and the use of heparin increases the "free" drug concentration by 30%, owing to heparin-induced activity of lipoprotein lipase, with resulting catabolism of lipoprotein. Thus, the concentration of free drug increases, leading to redistribution of the free drug into the erythrocytes.

If one avoids the above problematic blood-collection tubes, most evidence validates the use of either plasma or serum for measurement of TCA by LC, and serum for immunoassay. Based on my own experience with LC assay, EDTA-containing tubes provide plasma that does not yield an unidentified interference peak (with desipramine from serum). Before a blood-collection tube is used for a new antidepressant, a sizable number of subjects should be systematically studied, to establish its clinical efficacy.

III. Strategy of Designing an LC Assay

In the TDM of antidepressants, most tests involve the quantification of a known parent drug and possibly its demethylated metabolite. However, for regional and reference laboratories, the identity of the antidepressant may not be known. Also, emergency toxicology laboratories occasionally must analyze for an unknown number of antidepressants and metabolites. Thus the strategy will differ according to the category of clinical laboratory.

The first category is a typical hospital laboratory with moderate TDM need. If LC is chosen, the strategy should be based on simplicity, using two- to three-step traditional liquid–liquid extraction (as shown by Figure 1) or a solid-phase extraction column. LC analysis involves a reversed-phase or normal-phase column. Typically, five to 10 patients' specimens are analyzed within 2 to 3 h. By following these simple recommendations, any problems that emerge are identified readily.

The second category is the regional or reference laboratory. To measure large numbers of specimens, solid-phase extraction columns may be used, followed by LC analysis capable of simultaneously measuring many drugs and their metabolites. Inherently, the analysis time will be longer. To avoid peak mis-identification, it is important not to "over-use" the limited resolution of any given column. Thus, it would not be advisable to attempt simultaneous analysis of more than five or six drugs and metabolites, because of the inevitable loss of resolution, retention time changes, and interference from other drugs and metabolites. For increased specificity, the dual-wavelength or photodiode-array ultraviolet detectors would be useful for confirmation.

IV. Sample Preparation

Antidepressant concentrations are typically in the microgram per liter range, so the sample must be cleaned-up and pre-concentrated for detection by ultraviolet and other techniques. As outlined in the previous section, the choice of sample preparation depends on the number of samples, fewer or more than 10 (I would say), and on the mode of LC separation, reversed or normal phase. Further, serum or plasma is chosen as the sample according to the method to be used. The following discussion is classified according to the manual preparation—the "traditional" liquid–liquid extraction, solid-phase extraction, and the semi-automated and automated modes.

As emphasized above, reliable LC drug assay of antidepressants should be simple, enhancing recovery and reproducibility. Typically a two- to three-step procedure, with analytical recovery ranging from 60% to 80%, is preferred to four to five steps. Further, a simple procedure is more readily reproduced by various technologists in the same laboratory. Figure 1 shows the steps and selected chemicals used in a liquid–liquid extraction procedure. Typically, after an appropriate internal standard is added, we extract the alkalized serum or plasma with solvents such as n-hexane or n-heptane with added isoamyl alcohol to prevent formation of an emulsion, which would lower recovery. For extraction of polar metabolites, such as 2-OH-IMI and 2-
OH-DES, we use a more-polar solvent such as methylene chloride. After centrifugation, we transfer the organic phase for evaporation under nitrogen. Then, we reconstitute the extract in mobile phase or methanol for RP or NP analysis. Alternatively, we back-extract the organic phase with diluted acid for subsequent RP analysis.

To minimize TCA adsorptive loss, we use either silanized glassware or polypropylene tubes and pipettes. Recently, in a novel approach (27), a large amount of maprotiline was used, to minimize adsorption. Obviously, if the identity of the antidepressant is unknown, this procedure should not be used, lest maprotiline be present in the sample.

Since the late 1970's (28), clinical laboratories more and more have used solid-phase extraction techniques. For monitoring TCA, we activate cartridges containing a sorbent such as C-18 by sequential washing with methanol and water so that the functional groups extend from the silica surface. Then we add aliquots of serum or plasma sample, followed by internal-standard solution. To preferentially elute the interfering components while selectively retaining drugs/metabolites in the sorbent, we wash the columns with phosphate buffer. Afterwards, we use a stronger solvent, typically containing acetonitrile or methanol, to elute the retained drugs/metabolites. Recovery usually exceeds 90%.

If a large number of samples is to be processed, semi-automated and automated off-line preparation or automated on-line sample preparation is desirable. This should not be confused with the so-called automated LC, which does not mean automated LC for drug analysis. Potentially, truly automated methodology would include robotics and direct-sample-analysis, column-switching, micellar chromatography, and internal surface reversed-phase chromatography (29). Koteel et al. (30) showed off-line automated sample preparation, using Prep-1 with type W cartridge. Wong (29) reviewed the use of internal surface reversed-phase (Pinkerton) column for sample extraction and, with column switching, the sequential analysis of the eluate by use of an analytical CN column. This procedure may be automated by coupling to an autosampler and data processing unit. Ni et al. (31) described the use of the "Advanced Automated Sampler Processor" (AASP), with manual loading of sample into the reservoir, followed by automated extraction and direct injection into the analytical column. Bannister et al. (32) developed "FAST-LC", which automatically pipettes samples for organic extraction in the mixing coil, a scheme based on the Technicon technology. Inherent with automation, extraction by use of devices such as Prep-1, AASP, and others increases the cost. Thus, their merit would depend on the needs of the clinical laboratory. However, there is a trend to increasing use of solid-phase extraction columns, such as in the case of cyclosporine monitoring, which allows rapid extraction and clean-up that otherwise is not achievable (33).

V. Liquid-Chromatographic Column Variables

Because silica-gel-based columns are usually used for either reversed- or normal-phase analysis, the operating pH range of the mobile phase is restricted to the range 2 to 8, to minimize the dissolution of silica gel in acidic or basic medium. A small particle size of 3 or 5 μm offers better resolution, with higher back-pressure drop. The optimal flow rates for 5- and 10-μm particles are 1.5 and 2.0 mL/min, respectively.

For reversed-phase packing, other factors in addition to pH and particle size include functionality, ion-pairing, capillary, temperature, and carbon-load. The most versatile and reliable functional group, chemically bound to the silanol groups, is C-18, followed by CN, methyl, C-8, and phenyl. To minimize possible secondary interaction, the hydroxy groups not bound to functional groups should be capped chemically; this results in sharper peaks. Ion-pairing reagents, capable of forming less-ionic complexes with analytes such as tricyclics, increase retention. A high carbon-load column, with 20 to 25% instead of the normal 8 to 10%, increases retention—especially useful in analysis for polar metabolites. Finally, a separation at temperatures up to 70 °C hastens the interaction process, shortens analysis time, and gives sharper peaks.

VI. LC Assays of Antidepressants

Table 1 lists the drugs and selected extraction and analytical characteristics. Methods 1–15 outline the reversed-phase assays for the first-generation TCAs: AMI, NOR, IMI, DES, ND-DOX, and DOX and selected metabolites. Methods 16–21 show the normal-phase assays. Methods 15 and 22 to 38 list the analyses for alprazolam, amoxapine, bupropion, maprotiline, trazadone, and their selected metabolites. I have included selected assays prior to the 1980 review of Scoogins et al. (7), to highlight some historical perspectives, but the emphasis here is on those LC assays of antidepressants being used in the U.S. up to mid-1987.

For sample preparation of first-generation TCAs (no. 1–21 in Table 1), as pointed out in the previous section, most earlier extraction procedures follow the outline shown in Figure 1 with various organic solvents. Increasing use of solid-phase extraction column with high recovery is evident in more recent publications. Of particular interest are the automated procedures such as the FAST-LC (32) and the AASP (31).

After sample preparation, both RP and NP assays offer adequate precision (CVs 1.8 to 10%), sensitivity (1 to 25 μg/L), and analysis time (about 10 min). Ultraviolet detection at 254 nm is the most popular method, followed by 205 to 214 nm, while the use of fluorescence and electrochemical detections, though limited, offers equal or higher sensitivity and enhanced selectivity (47, 58). For simultaneous monitoring of parent drugs and metabolites, both demethylated and hydroxylated, the average analysis time for either normal or reversed-phase is usually longer, from 13 to 21 min. With the recent interest in the roles of hydroxylated metabolites, such as their contribution to side effects and their antidepressant potential (61, 86), their monitoring might become routine in the future.

In designing a simple RP LC analysis for the common TCAs, Wong and McCauley (18) and Breutzmann and Bowers (40) independently demonstrated analysis with a C-18 column, with use of elevated temperature and phosphate/acetonitrile as mobile phase. The latter authors also confirmed its clinical efficacy by comparison with GC/MS. Ion-pairing, without using increased temperature (35, 36) offers practicable alternatives. Thoma et al. (28) systematically evaluated blood-collection devices and pioneered the use of solid-phase extraction columns and the CN analytical column with minimum tailing. The elution profile is typical of reversed-phase; i.e., the parent drugs such as IMI and AMI are eluted before the more-polar demethylated metabolites such as DES and NOR. By using the CN column, Ni et al. (31) described the semi-automated analysis...
Table 1. LC Assays of Antidepressants

<table>
<thead>
<tr>
<th>No.</th>
<th>Drugs (ref.)</th>
<th>Extraction steps, solvent(s)</th>
<th>LC mode</th>
<th>Detection, nm</th>
<th>Analysis time, min</th>
<th>CV, %</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>RP assays for first-generation TCAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>AMI, NOR, IMI, DES, DOX, ND-DOX, TRI (6, 18, 34)</td>
<td>n-Hexane + IAA/dil ( \text{H}_3\text{PO}_4 )</td>
<td>RP (C-1)</td>
<td>254</td>
<td>9</td>
<td>3-7</td>
<td>0.5-3 ng</td>
</tr>
<tr>
<td>2.</td>
<td>AMI, NOR, metabolites (35)</td>
<td>n-Hexane/dil ( \text{H}_2\text{SO}_4 ) diisopropyl ether</td>
<td>Ion-pair (Partisil 5)</td>
<td>254</td>
<td>12</td>
<td>5-10</td>
<td>25 µg/L</td>
</tr>
<tr>
<td>3.</td>
<td>AMI, NOR, IMI, DES, DOX (36)</td>
<td>n-Hexane + IAA/dil HCI</td>
<td>Ion-pair (C-1)</td>
<td>254</td>
<td>13</td>
<td>2-7</td>
<td>2 µg/L</td>
</tr>
<tr>
<td>4.</td>
<td>AMI, NOR, IMI, DES, DOX, ND-DOX, PRO (28)</td>
<td>Clin-Elut™/dil HCl + MeOH/n-hexane/butyl chloride</td>
<td>RP (Spherosorb S5CN)</td>
<td>210</td>
<td>6</td>
<td>3-9</td>
<td>25 µg/L</td>
</tr>
<tr>
<td>5.</td>
<td>AMI, NOR, DES, IMI, metabolites (37)</td>
<td>n-Hexane + IAA/dil perchloric acid</td>
<td>RP (C-1)</td>
<td>254</td>
<td>14</td>
<td>6-8</td>
<td>5 µg/L</td>
</tr>
<tr>
<td>6.</td>
<td>AMI, NOR, IMI, DES, DOX, PRO (32)</td>
<td>“FAST-LC” Isooctane/1-propanol/dil ( \text{H}_2\text{SO}_4 )</td>
<td>RP (C-8)</td>
<td>205</td>
<td>8</td>
<td>5-8</td>
<td>5 µg/L</td>
</tr>
<tr>
<td>7.</td>
<td>IMI, DES (38)</td>
<td>n-Hexane + IAA/dil ( \text{H}_3\text{PO}_4 )</td>
<td>RP (Phenyl)</td>
<td>252 Ex 360 Em</td>
<td>15</td>
<td>2-15</td>
<td>1 µg/L</td>
</tr>
<tr>
<td>8.</td>
<td>AMI, NOR, IMI, DES (39)</td>
<td>MeOH + EtOAc n-hexane/dil HCl</td>
<td>RP (Micropak MCH-10)</td>
<td>210</td>
<td>14</td>
<td>4-8</td>
<td>25 µg/L</td>
</tr>
<tr>
<td>9.</td>
<td>IMI, DES, AMI, NOR (40)</td>
<td>n-Hexane + IAA/dil acid</td>
<td>RP (C-1)</td>
<td>215</td>
<td>11</td>
<td>7</td>
<td>25 µg/L</td>
</tr>
<tr>
<td>10.</td>
<td>TRI, DOX, ND-DOX, AMI, NOR, IMI, DES, MAP, PRO (30)</td>
<td>Manual: n-hexane Auto.: Prep-1 Type W cartridge</td>
<td>RP (µBondapak CN)</td>
<td>254</td>
<td>13</td>
<td>6.6</td>
<td>5 µg/L</td>
</tr>
<tr>
<td>11.</td>
<td>AMI, NOR, DES, IMI, DOX, ND-DOX (41)</td>
<td>n-Hexane + IAA/dil ( \text{H}_3\text{PO}_4 )</td>
<td>RP (Phenyl)</td>
<td>214</td>
<td>9</td>
<td>2.5-6.9</td>
<td>1 ng</td>
</tr>
<tr>
<td>12.</td>
<td>IMI, DES, Cl-AMI, ND-CI-AMI (31)</td>
<td>AASP</td>
<td>RP (Spherosorb CN)</td>
<td>252</td>
<td>8</td>
<td>4-5.6</td>
<td>10 µg/L</td>
</tr>
<tr>
<td>13.</td>
<td>2-OH-DES, 2-OH-AMI (42)</td>
<td>CH(_2\text{Cl}_2) + IAA/ phosphate</td>
<td>RP (ODS-2)</td>
<td>254</td>
<td>14</td>
<td>5</td>
<td>3 ng</td>
</tr>
<tr>
<td>14.</td>
<td>10-OH-AMI, 10-OH-NOR (cis and trans) (43)</td>
<td>n-Hexane + IAA/dil HCl</td>
<td>RP (C8 Spherosorb S5)</td>
<td>—</td>
<td>5</td>
<td>2 µg/L</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>AMO, 8-OH-AMO, DOX, ND-DOX, IMI, DES, AMI, and NOR (44)</td>
<td>Bond-Elut (CN phosphate/water/acetic acid + acetonitrile + n-butylamine/acetic acid)</td>
<td>RP (Zorbax CN)</td>
<td>254</td>
<td>17</td>
<td>3.8</td>
<td>8-10 µg/L</td>
</tr>
</tbody>
</table>

II. NP assays for the first-generation TCAs

<table>
<thead>
<tr>
<th>No.</th>
<th>Drugs (ref.)</th>
<th>Extraction steps, solvent(s)</th>
<th>LC mode</th>
<th>Detection, nm</th>
<th>Analysis time, min</th>
<th>CV, %</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.</td>
<td>AMI, NOR, IMI, DES (45)</td>
<td>n-Hexane + IAA</td>
<td>Normal (Silica B-5)</td>
<td>211</td>
<td>14</td>
<td>5-8</td>
<td>10 µg/L</td>
</tr>
<tr>
<td>17.</td>
<td>AMI, NOR, IMI, DES, DOX, ND-DOX (46)</td>
<td>n-Hexane + IAA</td>
<td>Normal phase (Varian SI-10)</td>
<td>254</td>
<td>8</td>
<td>2-10</td>
<td>15 µg/L</td>
</tr>
<tr>
<td>18.</td>
<td>IMI, DES, 2-OH metabolites (47)</td>
<td>n-Hexane + n-butyl alcohol</td>
<td>Normal phase (Silica B-5)</td>
<td>240 Ex 370 Em</td>
<td>21</td>
<td>2-4</td>
<td>1 ng</td>
</tr>
</tbody>
</table>

Abbreviations listed on next page.
### Table 1. (continued)

| 19. AMI, NOR, and OH metabolites (27) | n-Hexane + IAA | Normal-phase | 240 | 9 | 2.4–10.2 | 2.5–5 μg/L |
| 20. AMI, NOR, DOX, ND-DOX, IMI, DES, PRO (40) | Bond-Elut C18 methanol + ammonium acetate | Normal phase | 214/254 | 12 | 1.8–18.8 | 2–15 μg/L |
| 21. AMI, NOR, IMI, DES, DOX, Cl-IMI, MAP, PRO, and some metabolites (49) | n-Hexane + IAA | Normal phase | 214/254 | 15 | 10 | 10 ng/0.5 μL |

#### III. Assays for the second-generation TCAs and atypical antidepressants

| 22. Alprazolam (50) | Toluene + IAA/ acetonitrile + phosphate/n-hexane | RP (Bio-Sil ODS-10) | 202 | 10 | 4.8 | 1 μg/L |
| 23. AMO and 8-OH-AMO (44) | (Refer to no. 15) | | | | | |
| 24. AMO (6, 18, 34) | Ethyl acetate/ phosphate | RP (MC-18) | 214 | 22 | 3–5 | 1 ng |
| 25. AMO, 8-OH-AMO (52) | Bond-Elut C18 methanol + ammonium acetate | Normal phase | 254 | 6 | 10.8 | 3 μg/L |
| 26. AMO, 8-OH-AMO (53, 54) | Cln-Elut/1-butanol + hexane/dil HCl/1-butanol + hexane | RP (C-1) | 254 | 7 | 6–12 | 50 μg/L |
| 27. AMO, 8-OH-AMO, MAP (55) | n-Hexane + EtOAc or n-hexane + IAA | RP (Supelcosil-CN) | 211 | 11 | 2–5 | 10–25 μg/L |
| 28. Bupropion & metabolites (14) | n-Heptane + IAA | RP (LC-1) | 254/214 | 15 | 4 | 5–100 μg/L |
| 29. Bupropion & metabolites (15) | n-Hexane + IAA/dil H3PO4 | RP (MC-18) | 232 | 25 | 7.5 | 5 μg/L |
| 30. MAP, ND-MAP (51) | n-Heptane + IAA/dil H3PO4 | RP (C-1) | 214 | 10 | 8 | 3 μg/L |
| 31. MAP (30) | (Refer to no. 10) | | | | | |
| 32. MAP (52) | (Refer to no. 25) | | | | | |
| 33. MAP (48) | (Refer to no. 20) | | | | | |
| 34. TRA (56) | n-Hexane + IAA/dil H3PO4 | RP | 214 | 15 | 7.6 | 5 ng |
| 35. mCPP (TRA-metabolite) (57) | CH2Cl2 + IAA/dil H3PO4 | RP (MC-18) | 254 | 30 | 5.7 | 5 μg/L |
| 36. TRA, mCPP (58) | Methyl-1-butyl ether | RP (LC-1) | EC | 15 | 6.6 | 1 ng |
| 37. TRA (59) | n-Butyl chloride | RP (Ultrasphere C-18) | 242 | 6 | — | 50 μg/L |
| 38. TRA, mCPP (80) | CH2Cl2 + ethylene chloride + EtOAc + Na2SO4 | RP (Ultrasphere C-18) | 250 | 8 | — | 0.6–1.2 μg/L |

Nonstandard abbreviations (not listed in footnote 1, p. 848): IAA, isoamyl alcohol; 2-OH-IMI and -DES, 2-hydroxylmipramine and -desipramine; C-1, Bondapak C-18; Ex, excitation wavelength in nm; Em, emission wavelength in nm; UV, ultraviolet; EC, electrochemical detection; EtOAc, ethyl acetate.
for Cl-IMI, ND-Cl-IMI and DES, and Lensmeyer and Even-son (44) performed stabilized analysis with solvent recycling. Reece et al. (39) and Wong et al. (41) demonstrated LC analysis with an alternative column, phenyl; the latter authors explored its use in combination with x-onylamine mobile phase and ultraviolet detection for possible routine monitoring, and for dealing with interferences from (e.g.) phenothiazine and benzodiazepine. Bannister et al. (32) described automated LC analysis with a C-8 column, with an injection volume of 1 mL. By using higher carbon load columns, Wong et al. (42) could analyze for 2-OH-DES and 2-OH-IMI, and could do the more difficult separation of other antidepressants/metabolites.

In the normal-phase analysis, Vandemark et al. (45) pioneered the use of "polar" mobile phase with added base to minimize the ionization of TCAs, with resulting sharper peaks. To allow for possible silica gel dissolution caused by the small amount of base, they used a saturation column, connected between the pump and the injector. Sutfin et al. (49), using a similar mobile phase, in a simultaneous analysis for eight tri- and tetracyclic antidepressants and their demethylated and hydroxylated metabolites, showed that thioridazine and metabolite peaks do not interfere with analysis for DOX and ND-DOX.

In analysis for the "newer" antidepressants—AMO, 8-OH-AMO, bupropion and metabolites, MAP, ND-MAP, and TRA—both reversed- and normal-phase columns are useful, but analysis for some of their metabolites, such as 7- and 8-OH-AMO, mCPP, and bupropion metabolites, requires high-carbon-load columns. For example, 7-OH-AMO coelutes with 8-OH-AMO in some of the listed procedures, and mCPP, a metabolite of TRA eluting closely to an unknown peak. Both 7-OH-AMO and mCPP are found in low concentrations (57, 58), and may be of potential pharmacological interest. We advocate extreme care in their analysis. Although bupropion was recently introduced and withdrawn, the simultaneous analysis with some of its metabolites (14, 18) may be of interest in the event that this drug will be reintroduced later on as an antidepressant with specific indications.

VII. Drug Interference

Chromatographic interference from other drugs and metabolites erroneously increases the measured drug concentrations. In general, phenothiazines such as perphenazine, fluphenazine, and thioridazine and their metabolites, benzodiazepines, and other drugs have been shown to interfere in some procedures. Interference from previously non-interfering drugs/metabolites also could occur, owing to shortening of retention times as the column ages. In addressing this problem, we suggest an interactive approach, with use of the following guidelines, as recently proposed (7):

1. Use the appropriate blood-collection tubes. If not, repeat blood collection as soon as possible, or send the original sample to another laboratory with compatible methodology.
2. Review the patient's history, with emphasis on medication. If necessary, phone the therapist. Check the co-medication against the list of drugs interfering with the assay. Pay particular attention to the neuroleptics, benzodiazepines, and other drugs with similar tricyclic or tetracyclic structure.
3. After the analysis, check that the ratio of parent drug to demethylated metabolite is between 0.5 to 3.0, and that the total concentration is less than 500 μg/L. For sample concentrations exceeding these ranges, check the patient's history for potential interfering drugs, drug-to-drug interaction, and toxicity as a result of overdose.
4. For resolving potential drug/metabolite interference, use an alternative method, such as immunoassay, alternate chromatographic conditions, or use another chromatographic procedure, such as a phenyl column.
Figure 2 shows a successful resolution of interference by using a phenyl column, while Figure 3 shows that thioridazine co-elutes with the internal standard on a phenyl column. The rationale is based on the selectivity of the phenyl packing for the heterocyclic TCAs. Instead of the hydrogen bonding interaction of the analytes with the C-18 packing, the phenyl ring offers added selectivity via the mechanisms of cyclic interaction (67), π-π interaction (68), and other aromatic stacking interaction. Using the above approach, a six-month study shows the clinical efficacy of the phenyl column procedure (Table 2) (41). This shows that with the IMI/DES patient group, the phenyl column resolved some but not all of the interference. Thus other techniques such as immunoassays with monoclonal antibodies may be useful.

VIII. Conclusion

Since the late 1970s, various liquid-chromatographic methods have definitely enhanced TCA monitoring. TDM now may be useful for the therapy of patients treated with some of the first-generation TCAs, but the need to monitor other antidepressants awaits further studies. For toxicological emergencies, LC analysis identifies the drug and ascertains adequate drug elimination. With the advent of immunoassays using monoclonal antibodies for enhanced specificity, the role of LC may become complementary.

As much as LC and immunoassay technologies are available, survey data show that improvement is needed, and harmonization of TCA measurement may be an achievable goal. Automation may follow the route of robotics, the commercially available AASP and internal surface RP. Solid-phase extraction columns will continue to enhance the field of drug analysis, TCA monitoring in particular. Potential problems, such as drug interference, demand that the analyst be vigilant about the limitations of the procedure, and be aware of alternative procedures. The analyst should interact with the therapist for more meaningful interpretations of data on drug concentrations. Alternative methods such as immunoassays and procedures with other column packings are helpful. Emerging interest in the hydroxylated metabolites requires reassessment of monitoring strategy. Fluoxetine, a new antidepressant soon to be introduced, may be readily quantified by gas–liquid chromatography (69), and its routine monitoring awaits further study (70).

New and potentially useful analytical techniques include: novel packing, chiral separation, microcolumns, and super-critical chromatography.

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