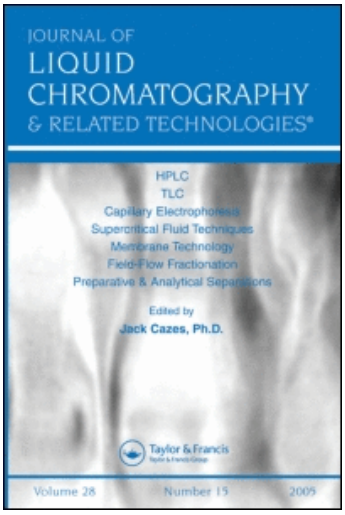

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Suckow, Raymond F.(1987) 'Problems in Sampling Techniques for Psychotropic Drug Assays', Journal of Liquid Chromatography & Related Technologies, 10: 2, 293 — 304
To link to this Article: DOI: 10.1080/01483918708066720
URL: http://dx.doi.org/10.1080/01483918708066720

JOURNAL OF LIQUID CHROMATOGRAPHY, 10(2&3), 293-304 (1987)

PROBLEMS IN SAMPLING TECHNIQUES FOR PSYCHOTROPIC DRUG ASSAYS

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ABSTRACT

The validity of analytical results in psychotropic drug assays is dependent upon, among other things, the proper method of specimen collecting and storage. This review cites the many problems associated with this aspect of analytical methodology including the proper collection tubes, anticoagulants and storage conditions necessary for the elimination of errors that may be introduced during sample handling. Specific examples are cited for the tricyclic antidepressant, antipsychotic, and some new antidepressant agents.

Recent advances in liquid chromatography have enabled the clinician and other researchers in the field of psychopharmacology to investigate the pharmacokinetics of psychotropic drugs and their metabolites. The introduction of smaller particle size packings for columns (3-5 microns), microbore high speed columns, and numerous bonded reversed phase columns in combination with mobile phases containing paired-ion reagents and/or amine modifiers have resulted in very powerful separation techniques. The recent introduction of an internal surface reversed-phase silica support column facilitates the direct injection of serum or plasma on

column, thus eliminating the time-consuming task of drug extractions from biological material and reducing the possibility of extraction procedure artifacts (1). In addition, a number of detection systems such as fluorescence, electrochemical and ultraviolet (fixed and variable wavelength) have added to the overall selectivity and sensitivity of the system. Newer detection systems such as simultaneous multi- wavelength or diode array UV detectors can determine peak purity as well as accurate and precise quantitation. These newer developments in liquid chromatography as well as in gas chromatography, mass spectrometry and radioimmunoassay procedures enables the analytical chemist to perform many psychotropic drug analyses with adequate precision, accuracy and sensitivity.

In spite of these achievements over recent years, there still remains an area of sample analysis which seems to be neglected or, at the least, underestimated in its contribution to the total analytical procedure. The technique in the collection of a sample from a patient (or other living animal) and the preservation of that sample until the actual time of extraction and chromatography is many times never specifically stated in published clinical studies or research articles. The type of collection tubes, anticoagulants (if any) and specific storage temperatures and other relevant information such as the use of antioxidants should be routinely stated in any research communication. This article, therefore, will discuss the problems relating to sample collection and storage as they pertain to the analysis of psychotropic drugs.

The groups of psychotropic drugs include the tricyclic antidepressants as well as most of the newer second generation antidepressants such as trazodone, maprotiline, bupropion, and others. The antipsychotic (neuroleptic drugs) such as the phenothiazines (chlorpromazine) and butyrophenones (haloperidol) will also be included in this discussion. These drugs all share several properties in common. They are all basic drugs and highly lipophillic. They are highly bound (85-95%) to blood proteins such as alpha$_1$-acid glycoprotein and lipoproteins, and to a lesser extent

albumin (2,3). This last property will become important when discussing the effects of various anticoagulants and collection tubes. Thus, the manner in which the sample is collected and stored will play a significant role in the final results which could alter subsequent therapy.

Although the clinical utility of routine serum/plasma monitoring of psychotropic drugs is questionable, there is certainly a need for accurate measurements when determining the pharmacokinetics, checking for compliance, investigations of the bioavailability of the same drug (i.e. generic vs trade name) and in situations where the patient is experiencing unusual side effects or does not respond to drug therapy. A recent report suggests that monitoring patients on tricyclic antidepressants may be more useful than for other psychotropic drugs since the data base for this class of compounds is more extensive resulting in a therapeutic range for some of these agents such as nortriptyline (4). These data have permitted a quicker response time for many problem patients, and, therefore, less cost to patients because of reduced time in hospitals or away from work.

BLOOD COLLECTION TUBES

The most popular method of blood collection is through the use of a vacuum collection system. The use of Vacutainers® (Becton Dickinson & Co.) prior to 1978 caused a lowering of total concentration of tricylic antidepressants in plasma (5-8). The plasticizer tris-butoxyethylphosphate (TBEP) in the container stopper displaced the basic drug from the alpha$_1$-acid glycoprotein in plasma thereby increasing the "free" fraction of the drug in plasma. This increase in the unbound fraction will redistribute between the red cells and plasma resulting in a decrease of the plasma drug levels (9). This decrease can range from 17 to 36% of a control plasma sample (all glass collection system) as reported in a study of the tertiary and secondary tricyclics amitriptyline and nortriptyline (10). Perel and Stiller (11) reported a decrease of 12% of the antipsychotic drug haloperidol using tubes with TBEP

stoppers as compared to TBEP-free stopper tubes. Since 1978, Becton Dickinson and Co. have prepared tubes specifically for the collection of basic drugs. Thoma et al. (12) concluded from their study of various tricyclic antidepressants in "new" and "old" Vacutainers that blood collection in glass syringes is unnecessary if the "new" Vacutainer is used. It is emphasized that only tubes specifically manufactured for therapeutic drug monitoring are free of TBEP. Despite the repeated reports in the literature concerning this interaction, samples still occasionally arrive at our laboratory in tubes with TBEP-containing stoppers through either ignorance or by the inadvertant selection of tubes.

There have been only a few studies concerning the effects of various anticoagulants on the levels of plasma psychotropic drugs. The most commonly used anticoagulants for plasma collection are heparin, ethylenediamine tetra-acetic acid (EDTA) and balanced oxalate (ammonium and potassium oxalates). One possible problem is the use of a heparin lock employed for multiple blood collection during pharmacokinetic sampling as well as individual routine sampling. Recent studies have demonstrated that as little as 50 units of heparin can cause a 30% increase in "free" drug concentration. The mechanism of action is believed to be the induction of lipoprotein lipase activity by heparin with the resulting degration of lipoprotein (13). Basic drugs bind to lipoproteins, and the resultant increased catabolism of these compounds increases levels of unbound drug. This could, therefore, lead to a redistribution of drug and, hence, a low plasma level since a greater proportion is distributed to the erythrocytes. Naranjo et al. (14) have suggested that this observation resulted at least in part from the fact that the subjects were in a non-fasting state which may be the primary determinant in activating the lipoprotein lipase. Pharmacokinetic data derived from such sampling techniques (indwelling heparin locks) must remain subject to doubt until further definitive studies are carried out (15). Evaluation of other studies regarding the effects of anticoagulants on plasma levels of psychotropic drugs is difficult because of a lack of suitable controls or insufficient statistical data (8,16,17).

Until recently, it was generally assumed that plasma and serum drug concentrations from the same subject were not significantly different. Stiller et al. (18,19) examined this relationship between various tricyclic antidepressants using EDTA as the anticoagulant. They found no significant difference between EDTA derived plasma versus serum levels of imipramine or desipramine. Spina et al. (20) also did not demonstrate a significant difference between serum and plasma tricyclics, although the serum levels were consistently lower in the 10 patients studied. Hullett et al. (21) compared serum and plasma concentrations of imipramine and desipramine in 10 patients using heparin as the anticoagulant for the plasma samples. Their findings show significantly lower levels in serum than in plasma. This study, however, may be subject to dispute since the tubes used to collect the blood for serum (Serum-Separator Tubes®, Becton Dickinson and Co.) have been implicated in artifactual lowering of the concentration of psychotropic drugs (16,22,23,24). A recent study conducted in part by our laboratory has revealed that plasma levels of the four major tricyclic antidepressants were indeed higher than the serum levels (25). Whole blood was collected in a 20 cc plastic syringe and transferred after needle removal into two separate 15 ml acid-washed borosilicate glass plastic screw-top test tubes. One tube containing balanced oxalate crystals to obtain plasma, and the second tube containing nothing for serum. After centrifugation, an aliquot of plasma or serum was transferred to plain tubes for storage at -20°C until analysis. Results are shown in Table 1.

TABLE I. Percent Tricyclic Antidepressant Plasma(P)-Serum(S) Differences. (With permission from Coccaro et al. (25))

	N	P-S(%)	Median	Range	Mean concentration ng/ml P	S
Imipramine	10	2.8±4.0%*	0.9%	-2.9-8.6%	79.5	76.3
Amitriptyline	10	6.8±2.4%**	7.0%	3.5-11.1%	97.3	90.7
Desipramine	20	15.0±8.8%***	14.9%	-7.7-30.5%	181.8	144.5
Nortriptyline	20	15.8±7.8%***	16.5%	0.0-29.8%	85.5	68.2

*P<0.1 **P<0.001 ***P<0.0001

Serum levels were about 15% lower for the secondary amine tricyclics (desipramine and nortriptyline), but considerably less (1-7% differences) for the tertiary tricyclics imipramine and desipramine. The differences observed here are apparently not a result of differential extraction recoveries between serum and plasma drug concentrations since spiking serum and plasma with these compounds result in similar recoveries. The authors suggested that the practice of using serum tricyclic levels be discouraged. There is a definite need for further studies along these lines which involve carefully controlled conditions such as using the proper anticoagulant and the elimination of serum-separator tubes as well as increasing the number of subjects for a more meaningful comparison.

SAMPLE STORAGE

Biofluids and tissues containing tricyclic antidepressants can be stored frozen for at least 6 months at -20°C (26). In our laboratory we have demonstrated that both tertiary and secondary amine tricyclic antidepressants are stable for more than 12 months if stored in tightly stoppered glass tubes. This property becomes important when considering batch processing of samples, thus reducing the overall cost of sample processing as well as eliminating day-to-day variance in clinical studies by simultaneously analyzing as many samples as possible. For long term storage, it is recommended that "frost-free" freezers not be used since the potential for freeze drying increases thereby altering the composition of the sample. Christiansen (27) reported that a possible source of error in the determination of the tricyclics is the property of adsorption onto glassware. This problem is particularly noticeable at low concentrations, and is not a constant factor. They reported a total loss of both imipramine and desipramine after 12 months storage at -20°C in whole blood. Silanization of the glassware or the addition of an analogous (but non-interferring) compound to

compete with the binding sites on the surface of the glassware are ways to avoid or minimize this problem.

Antipsychotic drugs may present more difficult problems since many phenothiazines are photosensitive and may require precautions such as collection in light shielded containers (28). Javaid et al. (29) suggest the use of an antioxidant in sample collection and storage of antipsychotic drugs because of their experience with trifluperazine. Chlorpromazine levels were observed to increase over storage time. This was believed to have been brought about by the reduction of chlorpromazine N-oxide to chlorpromazine (30). Friedel (31) suggested that the increased levels of chlorpromazine observed after storage was due to the reduction of the sulfoxide to chlorpromazine. Other investigators have reported the protection afforded by plasma both in preventing oxidation and absorption onto glassware during extraction procedures (32,33). Recent investigations into the problems of the stability of chlorpromazine in human blood have recently been reported (34,35). First, 10-14% of chlorpromazine was oxidized to chlorpromazine sulfoxide whether the blood was made alkaline with sodium hydroxide or with sodium carbonate before extraction. Additionally, chlorpromazine N-oxide appeared to be converted into a mixture of chlorpromazine and chlorpromazine sulfoxide in alkaline whole blood. Both reactions are reported to occur "instantaneously". These investigators concluded that chlorpromazine and its metabolites were remarkably stable in whole blood under physiological conditions. Davis et al. (36) showed increasing amounts of chlorpromazine when samples were stored below 0°C. They proposed that this increase was due to a conversion of a metabolite to the parent compound. However, these samples were extracted at a strongly basic pH and therefore the conversion of chlorpromazine N-oxide may have been an artifact of the extraction procedure rather than the storage procedure.

Recent reports have demonstrated that several new antidepressant compounds may present analytical problems with regard to their instability during improper storage. Nomifensine, a non-tricyclic

antidepressant is metabolized in man to several hydroxy and methoxy compounds as well as the N- and O-glucuronides (37). It has been demonstrated that the N-glucuronide of the unchanged parent compound is both acid labile and thermally unstable (38). Therefore, samples of nomifensine must be stored frozen and at a physiological pH otherwise the values of the drug will be falsely high (39,40). Recently, nomifensine has permently withdrawn form clinical use. Bupropion an FDA-approved but as of yet unmarketed antidepressant must also be handled judiciously. This compound is most stable at low pH's (2.5) and when stored frozen (-17°C) (41). Bupropion concentrations can decrease from 73-89% when maintained at pH 5-10 for 48 hours. Storage of bupropion in plasma at room temperature can result in a reduction in initial concentration of 55% after 48 hours. The three major basic metabolites of bupropion are not affected by these conditions.

Our laboratory has investigated the stability of nortriptyline in plasma shipped via regular U.S. Mail in the nonfrozen state (average time 2-4 days) versus the split sample shipped in dry ice. The results indicate comparable levels between the two samples. Other data on the stability of tricyclics at room temperature indicate that shipment in the unfrozen state is satisfactory. Handorf (42) reports that ideal storage temperature for tricyclics is 4°C, and that freezing these specimens results in sample deterioration and unreliable tricyclic levels. However, no data are presented.

The preservation of tissue samples in formaldehyde is a well-known procedure for long-term storage. However, toxicological specimens of secondary amine tricyclic antidepressants (desipramine and nortriptyline) stored in a formalin solution (10% formaldehyde in saline) will cause methylation to occur resulting in the formation of the corresponding tertiary amine (imipramine and amitriptyline) (43). This could have serious medico-legal implications if one were to rely upon the results from these specimens as opposed to fresh tissue analysis.

The object of this brief review has been to, hopefully, place a special emphasis on the importance of proper sample collection storage. It is by no means complete and, certainly, much controversy remains as to the "proper" way to handle these types of specimens. What is obviously lacking as one reviews this literature are carefully controlled studies involving the various types of collection tubes, anticoagulants and specific storage conditions with a sufficient sample size necessary to generate statistically significant results. Until these data are developed and accepted generally, it becomes the responsibility of each investigator to carefully evaluate the properties of the drug in question with respect to the above and include the results with any new analytical methodology. The best chromatographic precision and accuracy will have little meaning if the sample integrity prior to the analytical methodology is not validated.

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