ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
14 (1996) 1185–1190

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

# Bioanalytical strategies to support a discovery research programme[1]

Susan A. Rees*

*Upjohn Laboratories—Europe, Fleming Way, Crawley, West Sussex, RH10 2LZ, UK*

Received for review 13 September 1995; revised manuscript received 17 December 1995

**Abstract**

To facilitate the selection of drug candidates from a discovery research programme, a strategy was developed to assess the preliminary metabolism and pharmacokinetics of numerous chemical entities. A three-level "screening funnel" was set up, using both in-vitro and in-vivo techniques, requiring bioanalytical methods for the determination of parent compound. Simple high performance liquid chromatography (HPLC)/UV assays with minimal sample workup were adequate for the initial high throughput in-vitro screen used to assess in-vitro metabolic stability but a more selective sample extraction method was required for the second level of the screen. Here, rats were infused with drug to steady-state concentrations and whole blood, plasma, and brain tissue homogenate were analysed to assess clearance and to investigate blood/brain barrier penetration. Generally, HPLC/UV was adequate as only moderate sensitivities were required. However, the final level of the screen, a rat PO/IV bioavailability study, needed far more sensitive assays and often presented significant analytical challenges.

*Keywords:* Bioanalysis; Discovery research; HPLC; Metabolism; Pharmacokinetics

## 1. Introduction

The early investigation and evaluation of the pharmacokinetics and drug metabolism of novel chemical entities are playing an increasingly important role in the selection of drug candidates from drug discovery research programmes. In the late 1980s, The Upjohn Company, in collaboration with the Department of Pharmacology, University of Göteborg, Sweden, began investigating a series of structural analogues of 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH DPAT), a $5HT_{1a}$ agonist [1], for CNS indications (Fig. 1). Early studies had shown low oral bioavailability due to rapid metabolism; however, the large number of compounds to be studied precluded the general use of in-vivo methods. Consequently, a three-level "screening funnel" was proposed and set up using both in-vitro and in-vivo techniques, to assess the preliminary metabolism and pharmacokinetics of these structural analogues for which bioanalytical methods to determine the concentrations of parent compound were required.

* Tel.: (+44) 1293-531133; fax: (+44) 1293-548850.

[1] Presented at the 5th International Symposium on Drug Analysis, September 1995, Leuven, Belgium


*PII* S0731-7085(96)01764-5

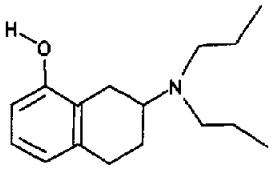

Fig. 1. 8-Hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH DPAT).

## 2. The screening funnel

Initial in-vivo studies on a structural analogue of 8-OH DPAT were supported by a high performance liquid chromatography (HPLC) method consisting of solid phase extraction (SPE) of the plasma samples followed by reversed phase HPLC with fluorescence detection [2]. To facilitate its application to analyse further analogues, the assay was well characterised during extensive method development. A range of SPE phases were examined, various HPLC columns and mobile phases were investigated and the detection parameters for a number of close analogues were explored. This "core" assay was then formally validated. The assay, with minor modifications to extraction, chromatography and detection, could then be logically adapted to support subsequent bioanalytcal activities from samples generated by the screening funnel [3].

The initial test system was a high throughput in-vitro assay where the metabolic stabilities of unknown compounds were assessed. Compounds were incubated in the presence of fresh hepatocytes, prepared using a modification of the collagenase perfusion technique [4,5], which are known to perform the appropriate metabolic processes seen in the liver in vivo. Acetonitrile was added to hepatocyte suspensions to stop the incubation and precipitate the proteins. After centrifugation, the supernatant was analysed for the disappearance of parent compound by reversed phase HPLC and the stability compared to that of a well-characterised structurally similar analogue. Additional preliminary information, such as the appearance of other drug-related peaks, was also acquired (Fig. 2). Those compounds with "good" metabolic stability were selected for further investigation.

The second test system used an in-vivo rat clearance model, which involves the continuous intravenous infusion of the compound to obtain a steady-state concentration. Plasma levels were quantified, the blood-to-plasma ratio measured and, in addition, brain tissue levels were assessed to investigate blood/brain barrier penetration. Again, the appearance of other drug-related peaks was noted. Selective and more sensitive methods

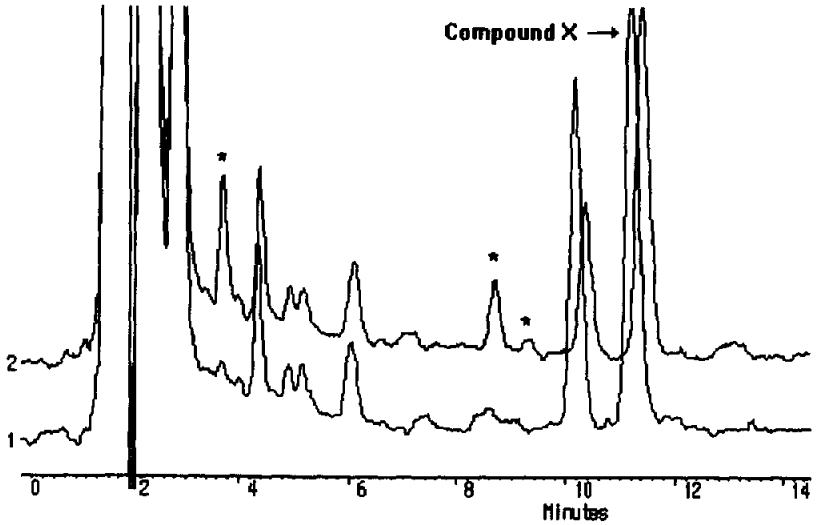


Fig. 2. Comparison of chromatograms of samples taken early on (1) and later (2) from the incubation media. The appearance of other durg-related peaks is shown (*).

Table 1
Typical analytical requirements for each test system

| Test system | Matrix | Typical no. of compounds examined per year | No. of samples assay | Desired sensitivity (ng $ml^{-1}$) |
|---|---|---|---|---|
| In vitro clearance | Precipitated isolated rat hepatocytes | 200 | 25 | 100 |
| In vivo clearance | Plasma<br>Whole blood<br>Brain tissue<br>Dose solution | 5–8 | 20 | 50 |
| Bioavailability | Plasma<br>Dose solution | 3–5 | 100 | 5 |

than those used for the in-vitro assay were needed ($< 50$ ng $ml^{-1}$), usually requiring more extensive method development. SPE methods were adapted from the "core" method to extract the compounds from the biomatrices, and usually, more efficient and robust chromatography was also required. An internal standard, a close structural analogue, was also included to minimise intra-assay variation. Adequate sensitivity was normally obtained with UV detection.

Those compounds determined as having low clearance and with favourable pharmacological properties were then assessed for oral bioavaila-bility. This was usually performed in the Sprague–Dawley rat, but other species, including beagle dog and Cynomolgus monkey, were also used. Sometimes, the assays developed for use in the rat clearance model could be applied to the analysis of plasma samples taken from bioavailability studies if the desired sensitivities (5–10 ng $ml^{-1}$) were readily obtained (early analogues were fluorescent). However, for compounds which possessed a poor chromophore or where matrix interferences occurred, further (and occasionally extensive) method development had to be undertaken.

## 3. Analysis for screening funnel

Initially, many compounds being evaluated were structurally similar and with minor adaption of the "core" assay, analogues could be assayed: reversed phase chromatographic methods could usually be adapted to determine unknowns from precipitated in-vitro hepatocyte suspensions; with small modifications, the "core" SPE method extracted unknown analogues from the biomatrices from in-vivo studies; detection parameters, such as UV maxima or fluorescence excitation and emission wavelengths, were often very similar. Analytical experience gained during the method development and validation of the "core" assay and its adaption and application for each new analogue led to the formation of a data base, information from which was applied to subsequent assays. A summary of analytical requirements for each test system is shown in Table 1.

## 4. Expansion of screening programme

Application of the "screening funnel" approach to candidate selection led to the expansion of the programme. The increase in numbers, the different structures and the changes in the physicochemical properties of these newer compounds

Table 2
In-vitro clearance screen

| | |
|---|---|
| Challenge | Increase from 3 to 6 compounds per day<br>New and diverse chemical structures<br>Limited resource |
| Strategy | Make maximum use of "data base" of analytical conditions |
| Approach | Compromise chromatographic parameters<br>—ideal $k'$ between 3–5<br>—actual $k'$ 2–7 (for example, see Fig. 3)<br>Use of alternative chromatographic modes<br>—ion-pair<br>—ion-exchange<br>Compromise on detection wavelength<br>—210 nm gives adequate response for most compounds with adequate selectivity |
| Outcome | Continue to provide 24 h turnaround<br>Continue to provide "preliminary" information on appearance of drug-related peaks (Fig. 2) |

Table 3
In-vivo clearance screen

| | |
|---|---|
| Challenge | New and diverse structureal classes<br>"Difficult" matrices i.e. brain tissue<br>Potential problems in obtaining adequate sensitivity<br>Limited plasma sample volume (0.025 ml) |
| Strategy | Aim for straightforward assay<br>Use of "data base" of analytical conditions |
| Approach | Optimise chromatography and detection parameters early on<br>Explore some SPE options<br>Limited assay "validation"<br>—linearity of extraction efficiency<br>—repeatability of extraction efficiency |
| Outcome | Relatively simple, robust assay<br>"Solid" data |

posed considerable analytical challenges. Some of these challenges for the in-vitro clearance test system are shown in Table 2. To facilitate the process, some compromises were made, for example in chromatography (Fig. 3) and in detection. However, wherever possible, analytical conditions were based on those used for similar analogues.

When compounds from a new structural class were assessed by the in-vivo clearance assay, a more detailed examination of assay conditions was undertaken (Table 3). For example, various chromatographic methods were explored to gain more selectivity from the extracts of biological matrices. Optimum detection conditions were also ascertained. Generally, the stability of an analyte

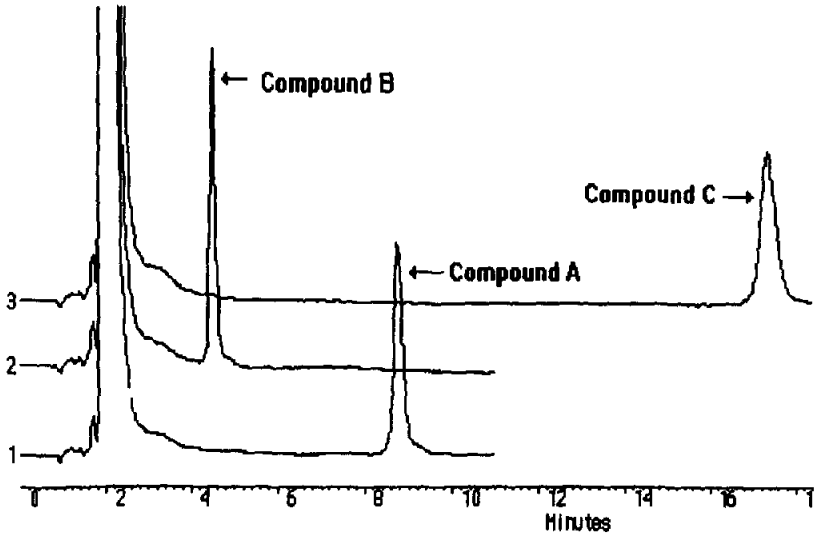


Fig. 3. Comparison of chromatograms of three structurally diverse standards assayed using identical analytical RP-HPLC conditions.

Table 4
Bioavailability Screen

| | |
|---|---|
| Challenge | High sensitivity required ($<10$ ng ml$^{-1}$)<br>Low sample volumes (0.025–0.1 ml)<br>New and diverse structural classes<br>Different detection modes<br>Data required quickly |
| Strategy | Simple, straightforward approach first<br>Method development activities run concurrently with other activities<br>Communication with "end user" of data/flexibility |
| Approach | Assess detection limits early on<br>Narrow bore chromatography<br>Simple sample preparation, i.e. precipitation<br>Pre- and postcolumn derivatisation<br>Lately, API-SCIEX<br>Limited "validation" |
| Outcome | Sensitive, specific and selective assays<br>Good quality data |

in a particular biofluid was not formally explored. A close inspection of the chemical structure for known instability-indicating groups, such as esters, or the presence of "leaving groups" identified those compounds requiring further investigation. However, where anomolous experimental data were obtained, indicating possible analyte instability, limited investigations to assess analyte stability in the biofluid or extract were done over the expected experimental period.

Some of the assays that had been developed for in-vivo clearance studies provided adequate sensitivity and selectivity for the analysis of plasma samples from the bioavailability studies. However, where the sensitivity proved to be inadequate, a wider range of analytical options were employed, including ion-exchange chromatography, pre- and postcolumn derivatisation methods, and electrochemical as well as UV and fluorescence detection (Table 4). More recently, LC/MS has been considered as an option for some compounds.

## 5. Conclusion

Some of the advantages and disadvantages in the bioanalytical approach for each of the screening systems are shown in Table 5. The rationale of the "core" assay and subsequent development of other assays has since provided analytical support for our "screening funnel" system for over 5 years.

Table 5
Summary of some of the analytical advantages and disadvantages for each test system

| Test system | Advantages | Disadvantages |
|---|---|---|
| In-vitro clearance | Minimum method development needed<br>Minimum sample preparation<br>Sensitivity no cirtical<br>Simple chromatography, i.e. RP-HPLC<br>Preliminary information on metabolism gained, i.e. presence/absence of other drug-related peaks | High resource requirements:<br>—equipment<br>—time<br>Diverse compound structure/assay may mean delay in results<br>More compounds can be incubated than analysed |
| In-vivo clearance | Some method development needed:<br>—recovery from biomatrices<br>—linearity<br>—detection<br>Sensitivity adequate | Method development can become extensive if problems occur with:<br>—poor selectivity<br>—poor chromophore<br>—novel structures |
| Bioavailability | Minimal sample preparation if selective detection available | Lack of sensitivity can mean extensive method development<br>Novel techniques/technologies |

## Acknowledgements

The author thanks colleagues within the Bioanalytical group, Dr. Neil Duncan and colleagues, Dr. Peter Daley-Yeats and colleagues, Doug Harris, and past and present industrial placement students.

## References

[1] L.E. Arvidsson, U. Hacksell, J.L.G. Nilsson, S. Hjorth, A. Carlsson, P. Lindberg, D. Sanchez and H. Wikstrom, J. Med. Chem., 24 (1981) 921–923.

[2] S.A. Wood, S.A. Rees and R.J. Simmonds, J. Liq. Chromatogr., 15 (1992) 1227–1248.

[3] S.A. Rees, S.A. Wood and R.J. Simmonds, in E. Reid and I.D. Wilson (Eds.), Bioanalytical Approaches for Drugs, Including Anti-asthmatics and Metabolites, Methodological Surveys in Biochemistry and Analysis, Vol. 22, Royal Society of Chemistry, Cambridge, 1992, pp. 65–70.

[4] P.O. Seglen, Exp. Cell Res., 82 (1973) 391–398.

[5] P.O. Seglen, Methods Cell Biol., 13 (1976) 29–83.