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Determination of serum and tissue levels of phenazines including clofazimine

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Abstract

A rapid and sensitive HPLC method is described for the analysis of synthetic phenazines, including clofazimine, from a variety of biological samples. Phenazines were extracted from serum, tissue and fat using a mixture of dichloromethane and sodium hydroxide. The drugs were then quantified on a reversed-phase C_{18} column using a mobile phase consisting of 594 ml of water, 400 ml of tetrahydrofuran, 6 ml of concentrated acetic acid and 0.471 g of hexanesulfonic acid. In this mobile phase, each phenazine tested had its own retention time. This allowed one phenazine to be used as an internal standard for the analysis of other phenazines. The method was validated for clofazimine [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(isopropylimino)phenazine] and B4090 [7-chloro-3-(4-chloranilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiperid-4-ylimino)phenazine] (VI) and shown to be accurate and precise across a broad concentration range from 0.01 to 50 μg/g (μg/ml). Extraction was 100% for each agent across this range. This system was used to measure clofazimine and VI levels following their administration to rats. The pharmacokinetic profile of VI was different to that of clofazimine, with high tissue concentrations but lower fat levels.

Keywords: Phenazine; Clofazimine

1. Introduction

Clofazimine (B663 or Lamprene) is used in the treatment of mycobacterial infections, especially leprosy [1]. Clinically it is very effective, but its use has a number of associated side effects [2]. Principally, clofazimine causes an orange–red discolouration of the skin of many patients receiving it and it can also induce gastrointestinal upset due to its deposition as crystals in the intestinal mucosa [3,4].

Chemically, clofazimine is a phenazine molecule and many other phenazines have been synthesised with the aim of reducing side effects and increasing efficacy [5–9]. Clofazimine and the other phenazines are both polar and strongly hydrophobic, making extraction and quantification from biological samples difficult. This has hampered pharmacokinetic evaluation of this group of agents, particularly that of clofazimine. As a result, there are no large scale reports of pharmacokinetic parameters, although some information has been produced from small scale studies [10–14].

Methods for the quantification of phenazines other than clofazimine are all based on a method developed by Barry et al. [15]. This method used an organic solvent-based liquid extraction with spectro-

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photometric quantification at the visible absorbance maximum. More recently, several sensitive methods have been developed for the quantification of clofazimine from serum and plasma samples. A thin-layer chromatography (TLC) method was described by Lanyi and Dubois [16] which used densitometry to measure extracted clofazimine. Several methods of analysis using high-performance liquid chromatography (HPLC) have also been published. The first method reported by Gidoh and Tsutsumi [17] used a reversed-phase C_{18} column with a mobile phase of tetrahydrofuran (THF) and 0.5% (v/v) acetic acid (40:60, v/v), with chloroform extraction from plasma and a limit of detection of 0.01 μg/ml. A second paper produced by Peters et al. [18] reported a similar limit of detection using a C_{18} column with a mobile phase of 0.0425 *M* phosphoric acid in 81% (v/v) methanol and an extraction system using chloroform–methanol. These methods are laborious and have inherent problems with precision and accuracy. A more accurate HPLC method was developed by Krishnan and Abraham [19]. These authors used solid phase extraction and an internal standard (I.S.) to quantify plasma levels of clofazimine. However, this method is unsuitable for measuring clofazimine in tissue matrices and its applicability to measuring other phenazines is unknown.

We have developed a method for extracting and quantifying all synthetic phenazines, including clofazimine. The phenazines tested were B749 [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(2-diethylaminoethylimino)phenazine] (I), B3640 [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(piperid-4-ylmethylimino)phenazine] (II), B3954 [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(N-carbobenzyloxyglycylpiperid-4-ylmethylimino)phenazine] (III), B3955 [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(N-glycylpiper-4-ylmethylimino)phenazine] (IV), B3976 [3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(N-phenylalanylpiperid-4-ylmethylimino)phenazine] (V), B4090 [7-chloro-3-(4-chloroanilino)-10-(4-chlorophenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiperid-4-ylimino)phenazine] (VI), B4100 [3-(3,4-dichloroanilino)-10-(3,4-dichlorophenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiper-4-ylimino)phenazine] (VII), B4103 [3-(4-trifluoromethylanilino)-10-(4-trifluoromethylphenyl)-2,10-dihydro-2-(2,2,6,6-tetramethylpiperid-4-ylimino)phenazine] (VIII) and B4154 [3-(3,4-dichloroanilino)-10-(3,4-dichlorophenyl)-2,10-dihydro-2-(3-diethylaminopropylimino)phenazine] (IX). This new method uses liquid extraction to quickly and simply extract phenazines from serum and tissue samples. The extracted phenazines have individual retention times in a HPLC system based on modifications of the three existing methods. As a result, a suitable phenazine is included in the system as an I.S., thus increasing accuracy and precision.

2. Experimental

2.1. Chemicals

All phenazine compounds used in this study, except clofazimine, were synthesised by Dr. O'Sullivan in the Chemistry Department of University College Dublin (Dublin, Ireland). Clofazimine was kindly supplied by Geigy Pharmaceuticals (Horsham, UK). All solvents used were of HPLC grade. Phenazine standards were prepared in dichloromethane (DCM) (LabScan, Dublin, Ireland).

2.2. Sample preparation

Serum and tissue samples were taken from rats which had been receiving clofazimine or VI at a level of 0.035% (w/w) in the diet over 26 days. Control serum and tissue samples were obtained from rats that had received no drug treatment. A 2-μg amount of an appropriate phenazine I.S. in DCM (VII for clofazimine analyses and clofazimine for VI analyses) was added to glass blood tubes (AGB Scientific, Dublin, Ireland). For standards and the variability studies, aliquots of the test phenazine were also added at this time. The DCM was evaporated off by heating to 40°C.

For tissue samples, 0.1 g of sample was homogenised in a 2-ml mortar, with a motor-driven revolving teflon pestle (AGB), in 1 ml of ultrapure water (Millipore, Bedford, MA, USA). The homogenate was poured into a glass blood tube coated with I.S. For standards and variability studies, blank homogenate was added to tubes coated with I.S. and standard amounts of the test phenazine. The remaining

homogenate was washed from the homogeniser tube with 1 ml of 5 *M* sodium hydroxide (NaOH). A 2-ml volume of DCM was added and each tube was mixed for 20 min on a blood tube mixer. Tubes were further mixed in a sonicating bath for 5 min. The tubes were centrifuged (Labofuge GL, Heraeus Instruments, South Plainfield, NJ, USA) at 1800 *g* for 15 min, resulting in an organic lower layer covered by a solid disc of tissue debris below the upper aqueous layer. A 1.1-ml volume of the organic layer was carefully removed to a glass autosampler vial (Chromachol, Trumbull, CT, USA) and allowed to evaporate off over 2 h by heating at 40°C on a heating block.

With serum, a 1-ml sample was added along with 1 ml of NaOH and 2 ml of DCM to I.S.-coated blood tubes. Extraction was then carried out as per the tissue extraction method outlined above.

For fat samples, 0.1 g of sample was homogenised as already described for tissue. After centrifugation, the upper aqueous layer was aspirated off and 1.5 ml of alcoholic sodium hydroxide (10%, w/v, NaOH in ethanol) added to the DCM layer. The tubes were then heated in a heating block at 80°C until bubbling stopped indicating complete evaporation of the DCM. A 6-ml volume of cold U.P. water and 2 ml of DCM were then added. The tubes were mixed for 5 min on the blood tube mixer and then centrifuged at 1400 *g* for 10 min. The aqueous soapy layer was aspirated off and the DCM layer was washed twice more in the same manner. A 1.1-ml volume of the DCM layer was removed and evaporated off in autosampler vials as described above.

2.3. HPLC quantification of phenazines

After extraction, samples were reconstituted in 60 μl of acidified THF (60 μl of acetic acid in 10 ml of THF). Fat samples were reconstituted in 120 μl of acidic THF. A 20-μl aliquot of the sample was loaded onto the HPLC column using an autosampler. Controls consisting of a mobile phase blank, a sample of the acidified THF used to dissolve the samples (at the start and end of the run), phenazine plus I.S. in the sample diluent and an extract from control tissue with no phenazines added, and one with the I.S. added were included with each chromatographic run. No peaks interfering with the test and I.S. phenazine peaks were evident from these controls. Measurement was performed using a Beckman System Gold HPLC system comprising a 507 autosampler, a 126 pump, a 166 UV detector and with data collected and analyzed by computer using version 8 of the Gold software (Beckman Instruments, Fullerton, CA, USA). The peak-height ratio (PHR) of analyte to I.S. was used to quantify drug concentrations from spiked standards. The concentration of phenazine present in a sample was measured using a plot of the log of the PHR versus the log of the concentration. Logarithmic scaling of the axes was necessary due to the broad range of the assay. The mobile phase consisted of 594 ml of ultrapure water, 6 ml of acetic acid, 400 ml of HPLC-grade THF and 0.471 g of hexanesulfonic acid (Romil Chemicals, Loughborough, UK). To reduce the likelihood of THF peroxidation, the solvent, purchased as required from the supplier, was used within a month of opening the bottle. Residual air was purged from the solvent bottle using a stream of nitrogen gas. The mobile phase was prepared immediately before use and was mixed and degassed in a sonicating bath for 5 min. The system operated by pumping the mobile phase through the column at a flow-rate of 1.5 ml/min, at ambient laboratory temperature (18–21°C) with the column eluent recycling into the mobile phase reservoir as part of a sealed recycling circuit of mobile phase flow. Sealing of the HPLC system was necessary because the retention time of a phenazines is very dependent on the THF content of the mobile phase. If THF evaporation were not prevented, the retention times would become very variable. Chromatographic separation was achieved on a Bondclone C_{18} reversed-phase (Phenomenex, Macclesfield, UK) column with dimensions of 300×3.9 mm I.D. and a particle diameter of 10 μm, with a C_{18} μBondapak cartridge precolumn. Absorbance of the column eluent was monitored at 285 nm. This wavelength was chosen because it was an average of the wavelengths at which all the agents gave their strongest absorbance.

3. Results and discussion

Two types of extraction method have been used previously with clofazimine or other phenazines. The solvent extraction method outlined by Barry et al. [15] and modified by others had posed several

difficulties for phenazine extraction. These included the need for large volumes of toxic organic solvents, with associated increases in measurement error, co-extraction of endogenous contaminants and an incompatibility with modern methods such as HPLC. The solid phase extraction method described by Krishnan and Abraham [19] is a significant improvement on previous procedures, since the use of an I.S. reduces the likelihood of errors in quantification. However, this method can only extract clofazimine in a liquid matrix, making it unsuitable for tissue analysis.

The HPLC mobile phases described by Gidoh and Tsutsumi [17] and Krishnan and Abraham [19] both used 0.5% acetic acid to increase the charge on the clofazimine molecule in solution and thereby to reduce the elution time. In our system, we have used a higher concentration of acetic acid, 1% of the aqueous content, with the ion-pairing agent hexanesulfonic acid, to improve the chromatography of the phenazines used in this research. All agents elute rapidly with quite narrow peaks and variability in retention time is reduced.

Table 1 shows the retention times of several of the phenazines tested. Fig. 1 shows the chemical structures of clofazimine, B4090 (VI) and B4100 (VII). Compounds VI and VII have similar retention times, due to their analogous chemical structures, and therefore clofazimine was used as an I.S. in the analyses of these compounds, while VI was used as the I.S. for clofazimine analysis. Fig. 2 shows representative chromatograms of clofazimine and VI extracted from serum and tissue samples.

Two representative agents, VI and clofazimine, were selected for inter- and intra-day and percentage recovery assays. Compound VI was chosen since it represents a new group of substituted phenazines which show the most promise therapeutically [8]. Clofazimine is the only phenazine with an established clinical profile. All other agents tested gave 100% recovery with linear responses from 50–0.01 μg/ml (μg/g) for serum and tissue samples and 100–0.02 μg/g for fat samples (results not included), although a large-scale verification of variability for each drug was not undertaken. Using this method, it has also been possible to measure the levels of phenazines found in the faeces following

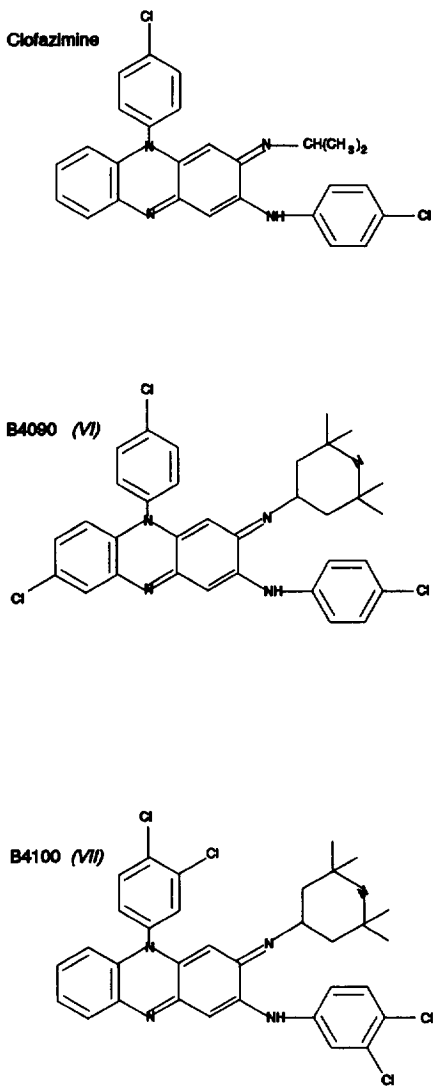

Fig. 1. The chemical structures of clofazimine, B4090 (VI) and B4100 (VII).

Table 1
The chromatographic retention time of some of the rimino-phenazine compounds analyzed

Compound	Retention time (min)	Compound	Retention time (min)
Clofazimine	5.6	B3976 (V)	3.2
B749 (I)	3.4	B4090 (VI)	4.4
B3640 (II)	2.7	B4100 (VII)	3.9
B3954 (III)	9.0	B4103 (VIII)	3.7
B3955 (IV)	2.6	B4154 (IX)	4.1

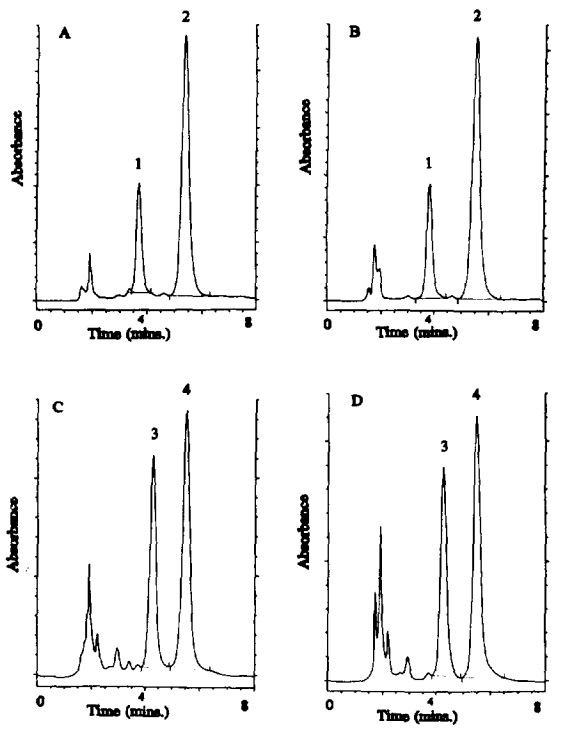

Fig. 2. Chromatograms of sample extracts. (A) Liver sample spiked with 5 μg of clofazimine and 2 μg of I.S. (VII). (B) Serum sample spiked with 5 μg of clofazimine and 2 μg of I.S. (VII). (C) Liver sample spiked with 5 μg of VI and 2 μg of I.S. (clofazimine). (D) Serum sample spiked with 5 μg of VI and 2 μg of I.S. (clofazimine). Peaks: 1=I.S. (VII) (3.9 min); 2= clofazimine (5.6 min); 3=VI (4.4 min); 4=I.S. (clofazimine) (5.6 min). The HPLC conditions were a Bondclone C_{18} column with a mobile phase consisting of 594 ml of ultrapure water, 6 ml of acetic acid, 400 ml of HPLC-grade THF and 0.471 g of hexanesulfonic acid, at a flow-rate of 1.5 ml/min and with the absorbance monitored at 285 nm.

oral administration. However, the co-extraction of endogenous compounds, thought to be porphyrins, reduced the limit of quantification to approximately 0.1 μg/ml.

3.1. Linearity and limit of quantification

With clofazimine standards from serum and tissue samples, the logarithmic plot of PHR versus concentration was found to be linear from 50–0.01 μg/ml (μg/g). For fat samples, the levels found were often in excess of this upper limit. Increasing the volume of the reconstituted sample from 60 to 120 μl produced a more suitable range of 100–0.02 μg/g. In the case of VI, the presence of a chlorine in the 7-position of the phenazine nucleus reduces its UV extinction coefficient. This reduces the limit of quantification for this compound to 0.02 μg/ml for serum or 0.02 μg/g for tissue samples, with the maximum limit remaining at 50 μg/ml (μg/g).

3.2. Percentage recovery

The recoveries of clofazimine and VI from serum, tissue and fat samples were determined across the linear range by dividing the peak-height ratios of extracted standards with those of paired unextracted standards. As shown in Table 2, Table 3 and Table 4, an average of 100% recovery was seen for clofazimine and VI across the linear range in serum, tissue and fat samples.

3.3. Accuracy and precision

To determine the intra-day accuracy and precision of the method, blank serum, liver and fat samples were spiked with clofazimine standards using an I.S. of 2 μg of VII, or VI standards using an I.S. of 2 μg of B663 (clofazimine). Five sets of standards were

Table 2
Recovery of clofazimine and VI from serum (n=5)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	102.8±24.7	24.0
0.02	95.2± 6.9	7.2
0.05	87.1± 9.4	10.6
0.2	94.4± 5.8	6.1
0.5	80.2± 8.2	10.3
2	96.3± 9.6	9.9
5	101.7± 2.8	2.8
20	104.2± 4.3	4.1
50	99.1± 1.4	1.4
VI		
0.02	95.4± 5.9	6.2
0.05	99.1± 6.1	6.2
0.2	103.2± 7.6	7.4
0.5	100.1± 3.2	3.2
2	96.9± 6.8	7.0
5	101.7± 3.9	3.8
20	102.5± 4.4	4.3
50	99 ± 1.3	1.4

Table 3
Recovery of clofazimine and VI from liver ($n=5$)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	97.6± 9.2	9.4
0.02	137.6±28.7	20.8
0.05	103.6±14.0	13.6
0.2	100.8± 2.2	2.2
0.5	89.4±12.4	13.9
2	98.4± 1.3	1.3
5	99.2± 5.4	5.4
20	100.1± 3.7	3.7
50	102.2± 5.0	4.9
VI		
0.02	95.2±10.3	10.8
0.05	118.6±12.4	10.5
0.2	106.2± 7.9	7.4
0.5	98.4± 1.3	1.3
2	97.9± 2.7	2.7
5	100.7± 1.7	1.6
20	99.6± 4.1	4.1
50	99.2± 3.2	3.3

prepared using the same stock concentrations. These standards were analyzed overnight in a single chromatographic run, with a set of calibration standards. The concentrations of the standards were determined

Table 4
Recovery of clofazimine and VI from fat ($n=5$)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.02	100.3± 5.5	5.5
0.05	101.8±10.2	10.1
0.2	102.0± 5.3	5.2
0.5	99.5± 9.0	9.0
2	96.8± 5.6	5.8
5	99.9± 4.0	4.0
20	97.2± 6.9	7.1
50	95.3± 5.17	5.4
100	100.0± 1.0	1.0
VI		
0.02	96.2±10.8	11.2
0.05	90.0± 3.2	3.6
0.2	93.7± 4.9	5.2
0.5	98.5±10.5	10.7
2	99.3± 1.5	1.5
5	101.0± 9.5	9.4
20	98.1± 2.9	3.0
50	115.0±15.0	13.0

Table 5
Intra-assay precision and accuracy for clofazimine and VI following extraction from serum ($n=5$)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	0.013±0.003	27.6
0.02	0.021±0.001	8.0
0.05	0.051±0.005	11.3
0.2	0.21 ±0.01	6.2
0.5	0.58 ±0.06	10.3
2	1.9 ±0.1	5.7
5	4.9 ±0.1	3.1
20	22.0 ±0.9	4.2
50	49.1 ±0.7	1.4
VI		
0.02	0.019±0.003	19.2
0.05	0.051±0.004	9.4
0.2	0.20 ±0.01	7.7
0.5	0.51 ±0.01	2.3
2	2.0 ±0.1	7.0
5	5.0 ±0.1	3.6
20	20.0 ±1.0	5.4
50	50.1 ±0.3	0.7

from the log plot of PHR to concentration of the calibration set. Table 5, Table 6 and Table 7 show the mean, standard deviation and % relative standard deviation (R.S.D.) of these spiked standards for both compounds in the serum, liver and fat during the intra-day analyses.

The inter-day accuracy and precision of this method were also determined for VI and clofazimine in serum, liver and fat samples. A set of spiked standards were prepared daily on five different days from freshly prepared stock concentrations, and the drug concentrations were determined from the log plot of PHR vs. concentration of a calibration set. Table 8, Table 9 and Table 10 show the inter-day mean, standard deviation and R.S.D. (%) of the spiked standard values for these analyses.

3.4. Pharmacokinetic studies

The method described in this paper has been used to study the tissue distribution of some of the therapeutically more promising phenazine agents. Table 11 shows a comparison of the levels of clofazimine and VI following their administration in the diet for 26 days at 0.035% (w/w). These results

Table 6
Intra-assay precision and accuracy for clofazimine and VI following extraction from liver (n=5)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	0.009±0.003	34.6
0.02	0.027±0.005	21.2
0.05	0.041±0.012	31.2
0.2	0.20 ±0.01	3.7
0.5	0.47 ±0.06	13.5
2	1.9 ±0.1	1.9
5	4.9 ±0.3	6.8
20	23.0 ±1.4	6.1
50	48.2 ±6.1	12.8
VI		
0.02	0.019±0.003	16.0
0.05	0.052±0.005	10.3
0.2	0.20 ±0.01	6.5
0.5	0.49 ±0.02	4.2
2	1.9 ±0.1	2.2
5	4.9 ±0.1	1.5
20	19.8 ±0.7	3.5
50	49.4 ±1.4	2.8

Table 7
Intra-assay precision and accuracy for clofazimine and VI following extraction from fat (n=5)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.02	0.017±0.002	13.2
0.05	0.053±0.007	14.7
0.2	0.19 ±0.01	5.8
0.5	0.46 ±0.04	10.6
2	1.9 ±0.1	6.6
5	4.9 ±0.2	4.1
20	20.0 ±1.4	7.1
50	50.2 ±2.8	5.5
100	99.3 ±1.0	1.0
VI		
0.02	0.020±0.001	9.5
0.05	0.051±0.003	6.5
0.2	0.21 ±0.01	2.8
0.5	0.50 ±0.01	2.0
2	1.9 ±0.1	4.1
5	5.0 ±0.1	2.5
20	21.0 ±0.7	3.5
50	50.3 ±1.1	2.2

Table 8
Inter-assay precision and accuracy for clofazimine and VI following extraction from serum (n=5)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	0.0097±0.000	6.3
0.02	0.019 ±0.006	35.1
0.05	0.054 ±0.009	17.1
0.2	0.20 ±0.01	7.7
0.5	0.52 ±0.01	2.6
2	1.9 ±0.1	8.4
5	4.7 ±0.1	3.5
20	20.6 ±1.1	5.4
50	49.7 ±0.6	1.3
VI		
0.02	0.021 ±0.002	11.6
0.05	0.046 ±0.009	19.7
0.2	0.20 ±0.01	3.2
0.5	0.49 ±0.02	5.7
2	2.0 ±0.1	6.0
5	4.9 ±0.1	1.5
20	23.0 ±1.6	7.3
50	48.6 ±3.4	7.1

Table 9
Inter-assay precision and accuracy for clofazimine and VI following extraction from liver (n=5)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.01	0.013±0.002	19.4
0.02	0.019±0.003	15.6
0.05	0.051±0.014	27.4
0.2	0.19 ±0.01	5.3
0.5	0.49 ±0.01	2.8
2	2.0 ±0.1	4.9
5	5.2 ±0.1	2.7
20	21.5 ±0.6	2.8
50	50.3 ±0.6	1.3
VI		
0.02	0.018±0.004	22.0
0.05	0.049±0.001	2.7
0.2	0.2 ±0.01	3.4
0.5	0.49 ±0.01	3.8
2	2.0 ±0.1	2.8
5	4.9 ±0.1	1.7
20	19.9 ±0.7	3.7
50	49.2 ±1.4	2.9

Table 10
Inter-assay precision and accuracy for clofazimine and VI following extraction from fat ($n=5$)

Concentration added (μg/ml)	Recovery (mean±S.D.) (%)	R.S.D. (%)
B663		
0.02	0.019±0.001	5.8
0.05	0.053±0.004	9.1
0.2	0.20 ±0.02	10.1
0.5	0.48 ±0.02	6.0
2	1.9 ±0.1	9.1
5	4.9 ±0.3	7.8
20	21.1 ±3.3	15.9
50	52.5 ±4.4	8.5
100	98.4 ±6.1	6.2
VI		
0.02	0.020±0.001	5.0
0.05	0.051±0.005	9.9
0.2	0.20 ±0.01	6.0
0.5	0.50 ±0.01	2.0
2	1.9 ±0.1	5.2
5	5.0 ±0.1	2.0
20	20.8 ±0.9	4.6
50	52.5 ±4.1	7.8

show that the distribution profile of VI is quite different from that of clofazimine. In the case of clofazimine, the highest drug levels are seen in the fatty tissue, with lower levels in all other tissues. However, with VI, the lowest levels of this phenazine are found in the fat. Although VI is more polar than clofazimine, it is still a very fat soluble agent. Why this phenazine is targeted away from fat tissue and more towards other tissues is not fully clear. However, we suspect that slight differences in the blood transport of these agents cause differences in the rate and specificity of uptake. A larger scale investigation of the pharmacokinetic properties of several of these agents has recently been accepted for publication in the International Journal of Leprosy (to be published). The reduction in the fat concentration, combined with the increased therapeutic efficacy of VI [8], suggest that this agent could be more active in the treatment of mycobacterial diseases, with fewer side effects.

Table 11
The average concentrations of clofazimine or VI found in biological samples following their administration for 26 days ($n=3$). The standard deviation (S.D.) of these values is also shown

Sample	Concentration of VI (μg/g)		Concentration of clofazimine (μg/g)	
	Mean	S.D.	Mean	S.D.
Fat	73.6	29.0	553.0	26.2
Spleen	1336.6	442.8	152.6	76.9
Liver	1384.0	323.9	81.7	17.4
Lung	669.4	243.6	146.1	15.8
Cerebrum	0.39	0.05	0.38	0.13
Serum	0.21	0.01	0.78	0.27

4. Conclusion

In conclusion, the method described allows the simple, sensitive and reliable measurement of synthetic phenazines, including clofazimine. This system is therefore useful both in routine clinical analysis and in directing the development of more efficacious phenazine antibiotics.

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