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Pyrolysis GC-MS Analysis of Auranofin Metabolites in Human Blood

Mohammad S Iqbal[1], Ahmed Muqeet[2], Maryiam Saeed[2], Saira Saeed[3] and Syed GT Kazimi[4]*

[1]*Department of Chemistry, Forman Christian College, Pakistan*

[2]*Department of Medicine, Allama Iqbal Medical College and Jinnah Hospital, Pakistan*

[3]*Department of Physiology, Lahore Medical and Dental College, Pakistan*

[4]*Department of Chemistry, University of Sargodha, Pakistan*

Abstract

Auranofin is an oral gold-based drug for rheumatoid arthritis. Its level in blood is determined by analysis of gold by atomic absorption spectrometry, which does not truly represent the intact drug. As a result till to date no correlation of gold level with efficacy or toxicity could be established. The object of this work was to determine levels of triethylphosphine and triethylphosphine oxide as alternate markers of the drug levels in body tissues. Pyrolysis GC-MS, being rapid and highly sensitive technique, was used for the analysis of the drug in human blood. This involved no extraction or preconcentration process. The method was found to be rapid (total analysis time 15 min), highly sensitive (limit of detection 1) ngmL^{-1} triethylphosphine oxide; 2) ngmL^{-1} triethylphosphine), reproducible (CV <2.3%) and accurate (>89% recovery). The method can be applied for this analysis to other body tissues.

Introduction

Chrysotherapy (treatment with gold salts) has been successfully used for treatment of Rheumatoid Arthritis (RA) with psoriasis for over 30 years [1,2]. Injectable gold salts along with the only oral compound auranofin, (2S, 3R, 4S, 5R, 6R)-3, 4, 5-triacetyloxy-6-(acetyloxymethyl) oxane-2-thiolate gold (I), have been used for this treatment. US FDA approval of methotrexate for RA in 1988 shifted the prescribing trend and use of gold salts started declining. The main reasons for reduced use of these drugs were:

1. Slow onset effect.
2. Frequent monitoring of gold level in blood for maintenance doses.
3. Less efficacy as compared with competitive drugs such as methotrexate. In addition to their use in RA, gold drugs are being extensively investigated as anticancer agents.

Recently, it has been demonstrated that auranofin is about ten times more active against parasites than metronidazole [3]. Thus there is a great future waiting for these drugs.

Monitoring of drug levels in body tissues has always been an analytical challenge. Most of the methods require extensive sample preparation steps, which affect the precision and accuracy. Similar challenge is being faced by analysis of auranofin. The drug is rapidly metabolized and the mechanism of metabolism is not exactly known; so in practice the released gold is measured to determine the level of the drug in human blood or tissues. After oral administration, about 25% auranofin (gold) is absorbed in blood through GI tract, of which 60% is bound by plasma proteins [4]. A mean blood gold level in humans is 0.68 ± 0.45 μgml^{-1} and the synovial fluid levels are about 50% of blood levels [3]. These data are based on gold levels determined by atomic absorption spectrometry. Nothing is known about the levels of triethylphosphine and thioglucose moieties although they play important roles in the efficacy of the drug. In the present work we report a pyrolysis GC-MS method for determination of auranofin level in body tissues by using triethylphosphine and triethylphosphineoxide as the drug signatures. This method requires little sample preparation.

Materials and Methods

Auranofin was a gift from GlaxoSmithKline, USA, n-hexane; triethylphosphine and triethylphosphine oxide were from Sigma-Aldrich, USA. Blank blood from healthy humans was

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Correspondence:
Mohammad S Iqbal, Department of Chemistry, Forman Christian College, Pakistan,
E-mail: saeediqbal@fccollege.edu.pk

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Table 1: Mass spectral data.

Compound	Formula	Mass	Fragments (m/z)
Triethylphosphine	$C_6H_{15}P$	118.16	C_2H_5 (29), $C_2H_5PH_2$ (62), $C_4H_{10}PH$ (90), M^+ (118.15)
Triethylphosphine oxide	$C_6H_{15}PO$	134.16	C_2H_5 (29), PO (47), C_2H_5PH (61), C_2H_5PHO (77), C_2H_5PHO (78), $C_4H_{10}PO$ (105), $C_4H_{10}POH$ (106), M^+ (134.15)

Table 2: Method validation data.

	LOD ($ngmL^{-1}$)	LOQ ($ngmL^{-1}$)	Precision (CV, %)	Accuracy (% recovery)
Triethylphosphine	1	3.3	Intraday: 1.5	89.7
			Interday: 2.1	
Triethylphosphine oxide	4	13.2	Intraday: 1.1	89.1
			Interday: 2.3	

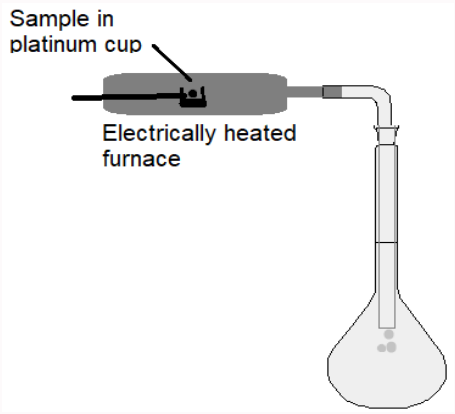

Figure 1: Pyrolysis setup.

obtained from a hospital and frozen in liquid nitrogen. The samples were stored at -60°C until analysis. The blood sample (500 µL) was transferred to a glass tube. To this, varying amounts (50 to 100 µL) of auranofin solution (10 $ngmL^{-1}$ in methanol) was added and homogenized for 30 s. The mixture was subjected to pyrolysis GC-MS analysis. Pyrolysis was carried out in the furnace of thermal analyzer SDT, Q-600 (TA instruments, USA). The pyrolytic products at 423-673 K(@ 20 K min^{-1} with 5 min hold time at 673 K) in a n-hexane (to make 10 mL) by use of an air-tight assembly after heating the samples (2.8 mg to 8.0 mg) in the thermal analyzer. The experimental setup is shown in Figure 1. This was an in-house assembly, which can be replaced with commercially available pyrolizers directly interfaced with the mass spectrometer. The solutions thus obtained were subjected to GC-MS analysis by using 1 µL injection in split less mode. The GC-MS system (Agilent Technologies, USA) consisted of: GC7890A gas chromatograph; MS5975C mass spectrometer with Triple-Axis detector; HP-5 MS column (30 m × 0.25 mm × 0.2 mm). The chromatographic and mass spectrometric conditions were: GC-Helium as carrier; flow rate 1.2 cm^3min^{-1}; injector temperature 230°C; column temperature 60°C for 0 min then 10°C min^{-1} to 300 for 5 min; ion source temperature 230°C; MSD transfer line 280°C; relative voltage 47 eV; mass range 50 to 600. Data were acquired and processed with the GC/MSD ChemStation. Compound identification was achieved by comparing the retention times with the standards and mass spectral library (NIST 05) of the GC-MS data system. Percent recovery, precision, limit of detection and limit of quantitation were determined. All the experiments were performed in triplicate and reported as the mean ± SD.

Results and Discussion

The blood samples spiked with auranofin produced total ion chromatograms and mass spectra as shown in Figure 2. The two peaks representing triethylphosphine (tr=4.0 min) and triethylphosphineoxide (tr=9.1 min) were observed, which were

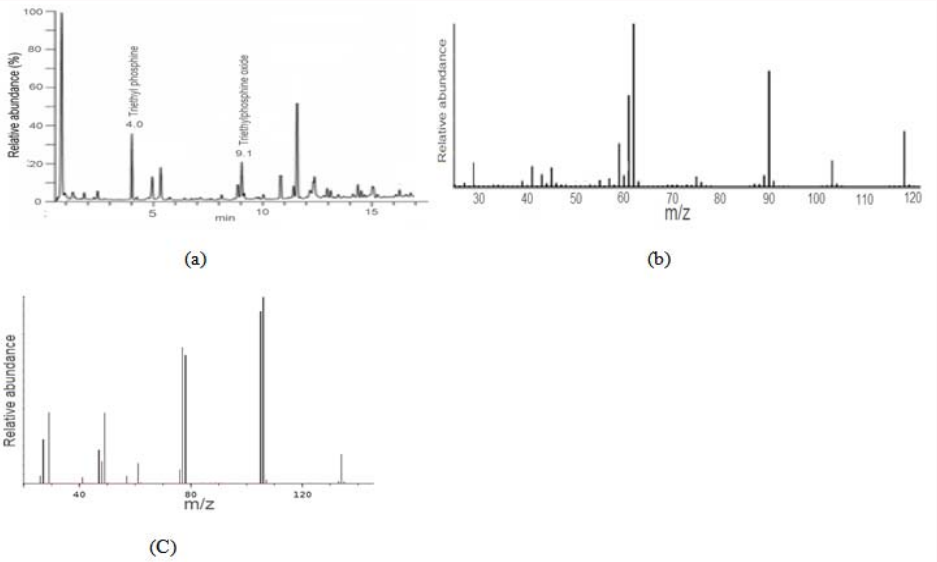

Figure 2: Total ion chromatogram (a) and mass spectra of triethylphosphine (b) tr=4.0 min and triethylphosphine oxide (c) tr=9.1 min.

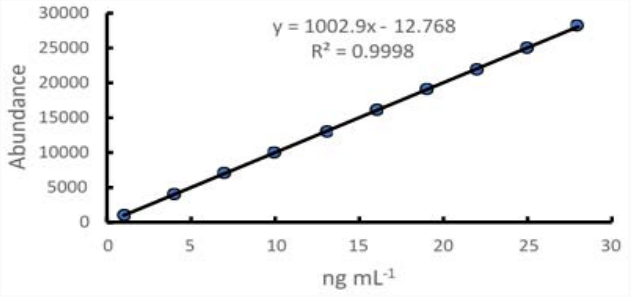

Figure 3: Linearity, limit of detection and limit of quantitation.

identified by their mass spectra (Figure 2). The mass spectral fragmentation data is presented in Table 1. It can be seen that triethylphosphine is eluted first followed by triethylphosphineoxide a difference in their boiling points; triethylphosphine boils at 127°C whereas triethylphosphineoxide boils at 243°C. The mass spectra of these species consist of all the expected fragments (Table 1) providing unequivocal identification. Triethylphosphineoxide is formed by oxidation of triethylphosphine.

Linearity, limit of detection and limit of quantitation

Linearity of response was observed (R^2=0.9998) in the concentration range under investigation (Figure 3). Limits of Detection (LOD) and Quantitation (LOQ) were determined from the linear curve by using the formulae: LOD=3.3 × STEyx/Slope and LOQ=3.3 × LOD, where STEyx is the standard error of standard error of the predicted y-value for each x in the regression. The method afforded very low values for these parameters (Table 2). This shows that the method is extremely sensitive so can determine the analytes (triethylphosphine and triethylphosphine oxide generated from pyrolysis of auranofin) at ultra-trace levels reliably. These results were validated by measuring the responses from standard triethylphosphine and triethylphosphine oxide.

Precision and accuracy

Intraday (repeatability) and interday (reproducibility) precision as determined by six replicates each at concentration levels near the LOQ (Table 2). Accuracy was determined in terms percent recovery, which was >89% in terms of triethylphosphine and triethylphosphine oxide. Precision in terms of CV was <2.3. Complete data are given in Table 2. These values demonstrate that the method is highly reproducible and accurate.

Real life sample analysis

A real-life blood sample obtained from a RA patient taking auranofin was analyzed by using the newly developed method. The values obtained were: triethylphosphine=130 ± 5.3 $ngmL^{-1}$, triethylphosphine oxide=55 ± 5.3 $ngmL^{-1}$ and auranofin (based on triethylphosphine)=0.65 ± 0.1 μgmL^{-1}.

Conclusion

Pyrolysis GC-MS provides for a rapid, precise, accurate, highly sensitive and rapid clinical method for determination of auranofin levels in blood through its signatures in terms of triethylphosphine and triethylphosphine oxide with little sample preparation. The method can be validated for determination of the drug concentrations in other body tissues.

References

1. Forestier J. Rheumatoid arthritis and its treatment with gold salts - results of six years experience. J Lab Clin Med. 1935;20(8):827-40.
2. Ragan C, Tyson TL. Chrysotherapy in rheumatoid arthritis. Am J Med. 1946;1(3):252-56.
3. Debnath A, Parsonage D, Andrade RM, He C, Cobo ER, Hirata K, et al. A high-throughput drug screen for Entamoeba histolyticaidentifies a new lead and target. Nat Med. 2012;18(6):956-60.
4. Gottlieb NL. Pharmacology of auranofin: overview and update. Scand J Rheumatol Suppl. 1986;63:19-28.