Research Journal of Chemical Sciences ISSN 2231-606X
Vol. **14(1),** 1-7, February **(2024)** *Res. J. Chem. Sci.*

Quantification of dapsone in human plasma by using UPLC-MS/MS technique

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Available online at: www.isca.in, www.isca.me
Received 20th June 2023, revised 5th July 2023, accepted 28th December 2023

Abstract

To validate the method for determination of dapsone in K_2EDTA human plasma using high performance liquid chromatography method with tandem mass spectrometry. Bioanalytical method was developed at Bioanalytical Research of Synergen Bio Pvt. Ltd. and validated as per method validation SOP. 0.200mL plasma was aliquoted and 0.050mL of internal standard dilution was added to it, except in blank in which 0.050mL of diluents was added and vortexed all the samples. 0.200 mL of 5mM Ammonium Acetate was added to all samples and vortexed for few seconds. Conditioning and equilibration were done with 1 ml of Methanol. Equilibration with 1ml of HPLC Water. Samples were loaded on Cartridges and washing with 1 ml HPLC water then by 5% MeOH in HPLC water. Samples were eluted with 1ml elution solution (70:30: Acetonitrile: 5mM Ammonium Acetate Solution). Transfer the samples into pre-labelled autosampler vials. Linearity of Calibration standard were linear in range of 5.000–3000.000ng/mL for Dapsone. The method validated at current regulatory requirements for sensitivity, selectivity, accuracy and precision, linearity, matrix effect, autosampler carryover effect, cross reactivity and stability of bench top, freeze thaw, autosampler and stability in whole blood etc. However, Recovery for dapsone and its internal standard were found precise, consistent and reproducible. (i.e. not more than 100%). The validated method has been used for the quantification dapsone in Human plasma and can be applied to BA/BE studies of dapsone.

Keywords: LC-MS/MS, Validated method, Dapsone.

Introduction

Clinical Pharmacology: Metabolism: The other main metabolic process that produces hydroxylamine dapsone is hydroxylation, which may be the cause of the hemolysis associated with methemoglobinemia caused by dapsone. The main metabolite, monacetyldapsone, as well as various mono- and diacetyl derivatives are formed when dapsone is acetylated. There is genetic variation in acetylation.

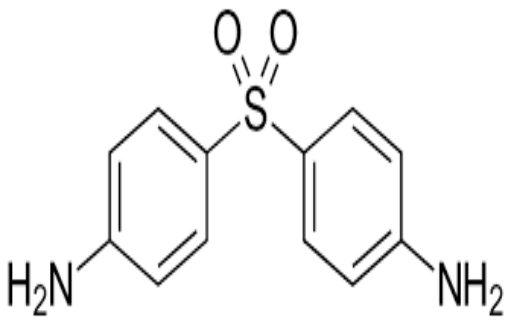
Pharmacokinetics: Absorption: Peak plasma concentrations of dapsone occur 2 to 8 hours after a dose and are almost entirely absorbed from the digestive system. Dapsone's plasma protein binding ranges from 50 to 80% and it is over 100 percent for its monoacetylated metabolite. The MIC for M. leprae is considerably exceeded by dosages of 100mg daily, which provide trough values of 0.5 micrograms/mL before steady-state concentrations are reached after at least 8 days of daily dosing.

Excretion: Only 20% of a dose of dapsone is eliminated as an unaltered medication, most of it in the urine.

Distribution: The half-life is between 10 and 80 hours long. Dapsone is recycled in the enterohepatic system. It is widely dispersed, appears in breast milk and saliva, and passes the placenta.

Indications: Dapsone Tablet 100mg are indicated for the treatment of dermatitis herpetiformis, leprosy and actinomycotic mycetoma.

Mechanism of Action: Dapsone (Figure-1) is a sulfone that has broad antibacterial activity but is most commonly used to treat Mycobacterium leprae. Additionally, it is effective against Pneumocystis carinii and Plasmodium. P-aminobenzoic acid, like the sulfonamides, inhibits antibacterial action. It works through a similar mechanism to sulfonamides, which includes preventing the synthesis of folic acid in sensitive species. Although it may potentially have modest bactericidal activity, it is typically thought of as being bacteriostatic against M. leprae[1-10].



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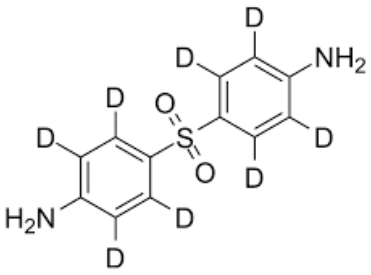

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Figure-1: Structure formula of (I) Dapsone and (II) Dapsone D8.

A bioanalytical approach that was validated to cover the study concentration was used because Dapsone comes in a variety of dosage forms that are dosed in varied strengths. Few techniques have been established on the rat plasma matrix, according to a survey of the literature. A few bio-analysis techniques, including ultra-high-performance liquid chromatography with UV detector[1,2] and liquid chromatography-mass spectrometric (UHPLC-MS)[3–7], have been published for quantifying dapsone in human plasma K_2EDTA as an anticoagulant. K_2EDTA was used as an anticoagulant in this study's investigation, and a sensitive, sample-based, quick, and specific LC-MS/MS approach was reported. This method was suitable for estimating dapsone's bioavailability and bioequivalence and encompassed the drug's Cmax range. To date, only one method has been published for measuring Dapsone levels in human plasma[8–14]. In the current study, we have created and validated a bio-analysis method with a short run time using a straightforward solid phase extraction procedure to obtain tidy samples and prevent matrix effect. The proposed bioanalytical approach has the following benefits over those previously reported: i. The amount of sample that needed to be taken from a person at a given time point for the study analysis was greatly decreased. This enables the inclusion of additional time points for sample collection; ii. as applied bioanalytical methods were extraction techniques optimised with solid phase extraction proven to be effective procedures with little matrix effect and sample cleanliness iii. Employing an internally labelled standard that is physically and chemically analogous to the analyte dapsone minimises internal standard variance and batch failures. The mentioned details, the use of labelled internal standards, solid phase extraction, the requirement for a small sample aliquoting volume, and the 3.0minute run time are all important. Dapsone is quantified in human plasma using the approved method and the LCMS/MS technology matrix.

Materials and Methods

Chemicals and reagents: Working standard of Dapsone and Dapsone D8 was received from clear synth labs., Acetonitrile of HPLC, UHPLC and Spectrophotometry grade was obtained from J.T. Baker. Water from Finar HPLC Grade. Methanol from Thermo Fisher Optima ACS Grade and Ammonium acetate obtained from Biosolve ULC/MS-CC/SFC Grade.

Instrumentation and chromatographic conditions: Chromatographic separation shall be carried out by using, Phenomenex, Luna 100*4.6mm 3µm. Mobile Phase Mobile Phase (Acetonitrile: 5mM Ammonium Acetate: 50:50, v/v). The retention time of Dapsone and Dapsone D8 are approximately 1.45±0.5, and 1.45 ± 0.5 Minutes respectively. The overall run time is 3.00 minutes. The UHPLC system was coupled to a detector conditions for LC-MS/MS- 4500 triple–quadmass spectrometer from Sciex installed with turbo spray ion source in positive polarity electro spray ionization (ESI) technique. Resolution Q1 and Resolution Q3 an unit. The turbo ion-spray with positive mode source parameter optimize as Curtain (CUR) 40; CAD 8; IS 5500; TEM 550; GS1 40; GS2 60 were as compound parameter as DP 80; EP 10; CE 20; CXP 10 for dapsone and dapsone D8 respectively using Dwell time 300 msec. Analyte and IS optimize in MRM mode using precursor and product of Dapsone, m/z 249.000→156.100 Dapsone D8, m/z 257.100→160.00.

Data Processing: Acquire chromatograms using the computer-based Analyst software (version number 1.7.1) supplied by Sciex. Unknown samples conc. is calculated from the following equation using regression analysis of the spiked plasma matrix calibration standard with the reciprocal of the square of the drug conc. weighing factor (1/concentration* Concentration i.e. $1/X^2$) for analyte. Acquired data shall be reviewed using Analyst software 1.7.1 version or higher version.

Y=mx+b, x= Analyte concentration, m= calibration curve slope, Y= Analyte to internal standard peak area ratio, b= calibration curve y-axis intercept.

Stocks and working dilution solutions: Weigh dapsone working standard approx. 5.0mg and transfer into a 5.0mL of vol. flask and dissolve with methanol and make the volume up to the mark with same to get final conc approx (1000ppm) and store the stock solution at 2-8ºC. Weigh about 1mg of Dapsone D8 working standard and transfer into a 1.000mL of volumetric flask. Dissolve in its methanol and make up the volume with the same to produce a solution of about 1mg/mL of concentration of dapsone D8. Store the stock solution at 2-8ºC.

Dapsone stock solution for calibration and quality control samples was separately preparedat the concentrations of 1mg/ml. Prepare the Working solution of Dapsone concentration range of 0.125µg/ml to 75.140µg/mL for by using diluent solution methanol: water (50:50, v/v). Prepared internal standard dilution of Dapsone D8at a conc. of 3000ng/mL using diluent methanol: water (50:50, v/v).

Calibration and quality control samples spiked in human plasma matrix: Linearity samples of dapsone was prepared at the conc. Level of 3000.000, 2400.000, 1800.000, 1200.000, 500.000, 250.000, 10.000 and 5.000ng/mL by spiking working dilution solutions in 0.200mL blank plasma matrix and at different levels QC samples were prepared at of LLOQQC,

LQC, M1QC, MQC and HQC at conc. Level of 5.000, 12.000, 300.00, 1200.00 and 2400.00ng/mL in 0.200ml blank plasma matrix. Aliquot 0.300mL of each spiked CC standards and QC's into pre-labelled Ria vials and stored in -70^{0}C (±10^{0}C) deep freezer until analysis. Also required aliquots of LQC, HQC and DQC sample (If required) shall be stored in freezer -20^{0}C (± 5^{0}C) and -70^{0}C (±10^{0}C) for long term stability.

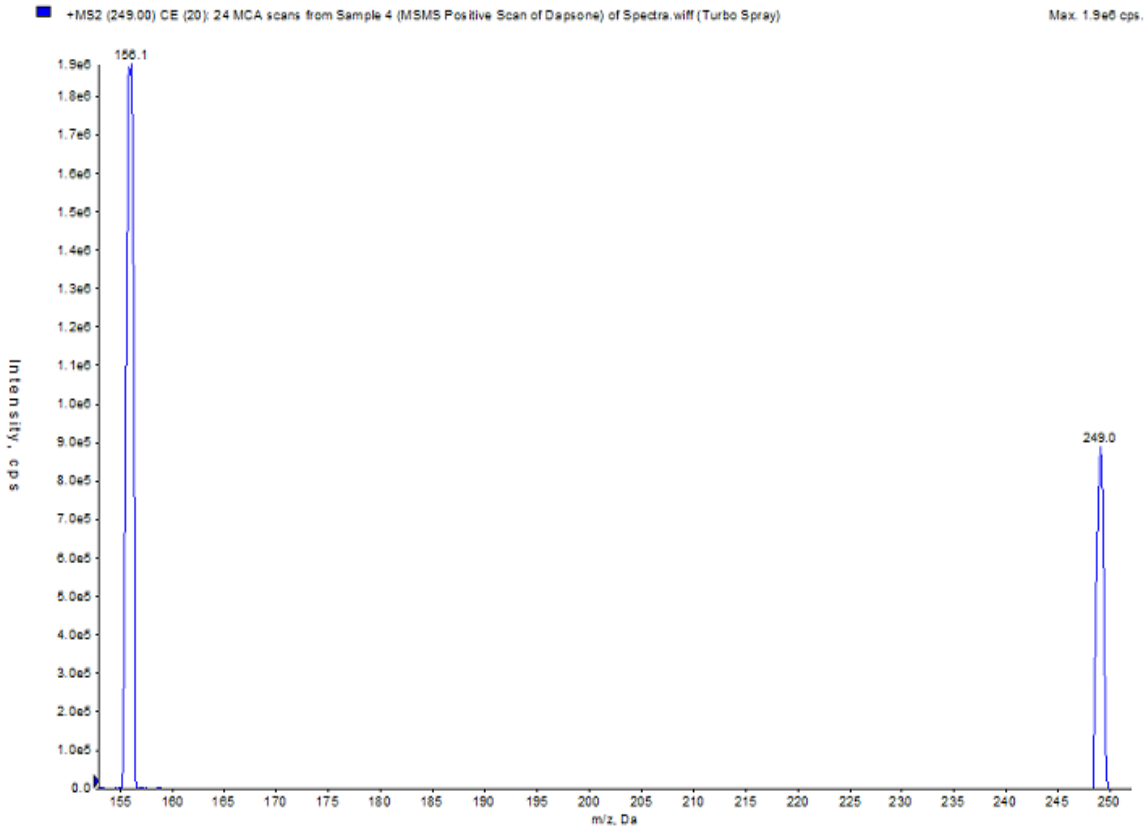
Sample preparation: Retrieve the CC and QC samples with subject samples (in case of project sample analysis) from the freezer -70^{0}C and kept at RT to thaw the samples and arrange the sample as per batch sequence. Vortex the samples to check complete mixing.

Aliquot 200µl of Plasma sample in to pre-labelled Ria vial. Add 50µl of internal standard dilution solution 3000.000ng/ml for Dapsone D8 to each pre-labelled Ria vial except blank sample and add 50µl of diluent solution in blank sample vortex for few second. Add 200µl of 5mM Ammonium Acetate solution into all samples and vortex for few seconds.

Use SPE Method using following steps: Orochem Panthera Deluxe 30mg, 1ml Cartridges. Conditioning: 1ml of MeOH Equilibration: 1ml of HPLC Grade Water. Load the entire samples on Cartridges. Samples wash with 1ml Ultra-Pure Water then by 5% methanol in Water. Elute the Samples with 1.000ml of elution solution (70:30:Acetonitrile:5mM Ammonium Acetate). Transfer the samples into pre-labelled auto sampler vials. Note: Sample preparation procedure shall be carried out under monochromatic light.

Results and Discussion

Method Development: Selectivity No Interference observed less at analyte RT and at IS RT with respective LLOQ area response in all 10 different lot of human plasma samples. Sensitivity Mean % Nominal: 98.984% CV: 4.81 Signal to noise ratio was ≥ 5.00. Precision and Accuracy (Quality Control Samples) QC Coefficient of variation: 2.63 to 6.49% QC% Nominal value 97.859% to 106.185%. Recovery Mean Analyte Recovery: 64.8713% Mean Internal Standard: 86.197%. Matrix Effect Mean Internal Standard Normalized Matrix Factor is 0.85075 for LQC and 0.997.53 for HQC Coefficient of variation of IS Normalized Matrix Factor 2.82 for LQC and 1.31 for HQC. Bench top Stability QC% Change value -8.442% for LQC and 6.516% for HQC. QC Mean % Peak Area Ratio is 98.4494% for LQC and 108.3961% for HQC.



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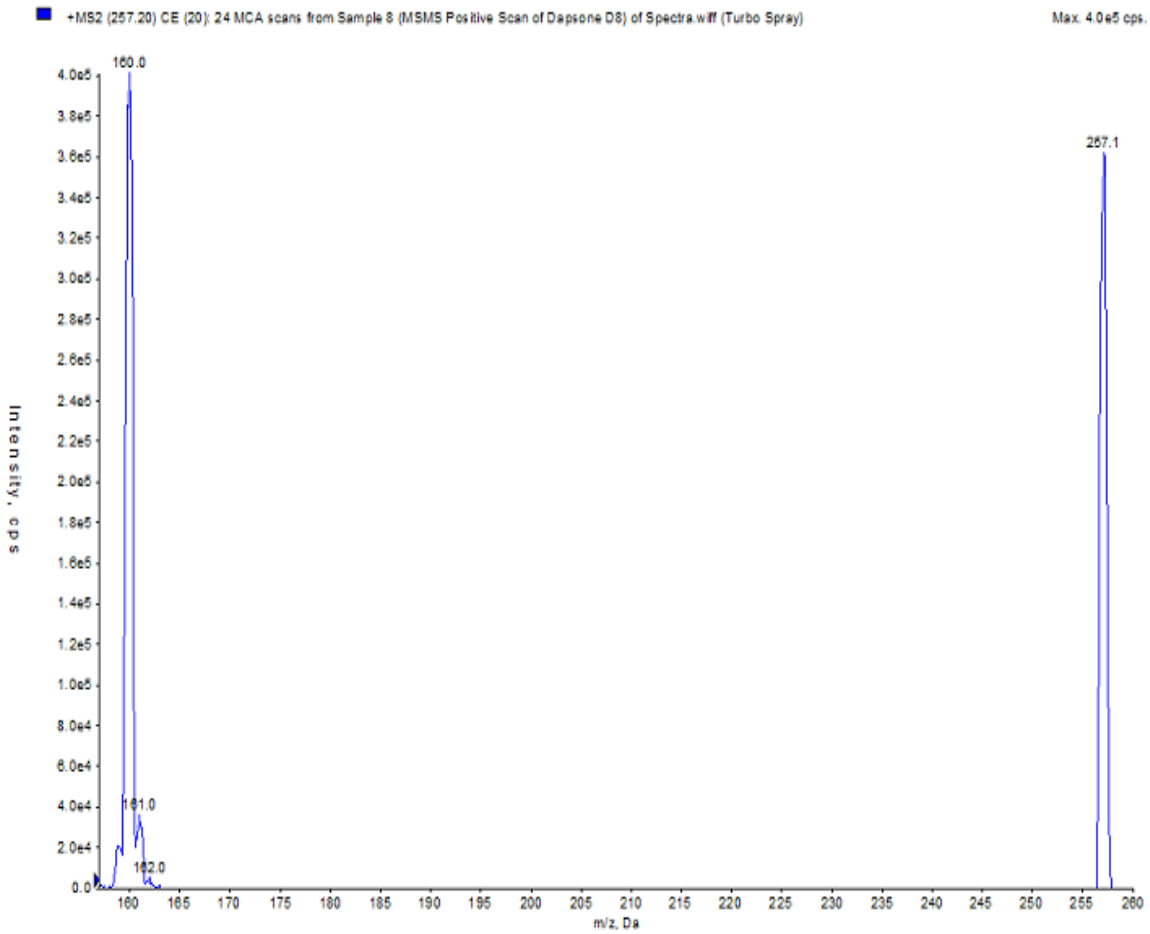

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Figure-2: (I) Dapsone and (II) Dapsone D8 MSMS Positive Scan

Method Validation Parameter: Selectivity and Specificity: Selectivity Interference at Analyte RT: ≤ 0.00%, Interference at IS RT: ≤ 0.00%. Cross Reactivity Interference at Analyte RT: ≤ 6.49%, Interference at IS RT: ≤ 0.00%.

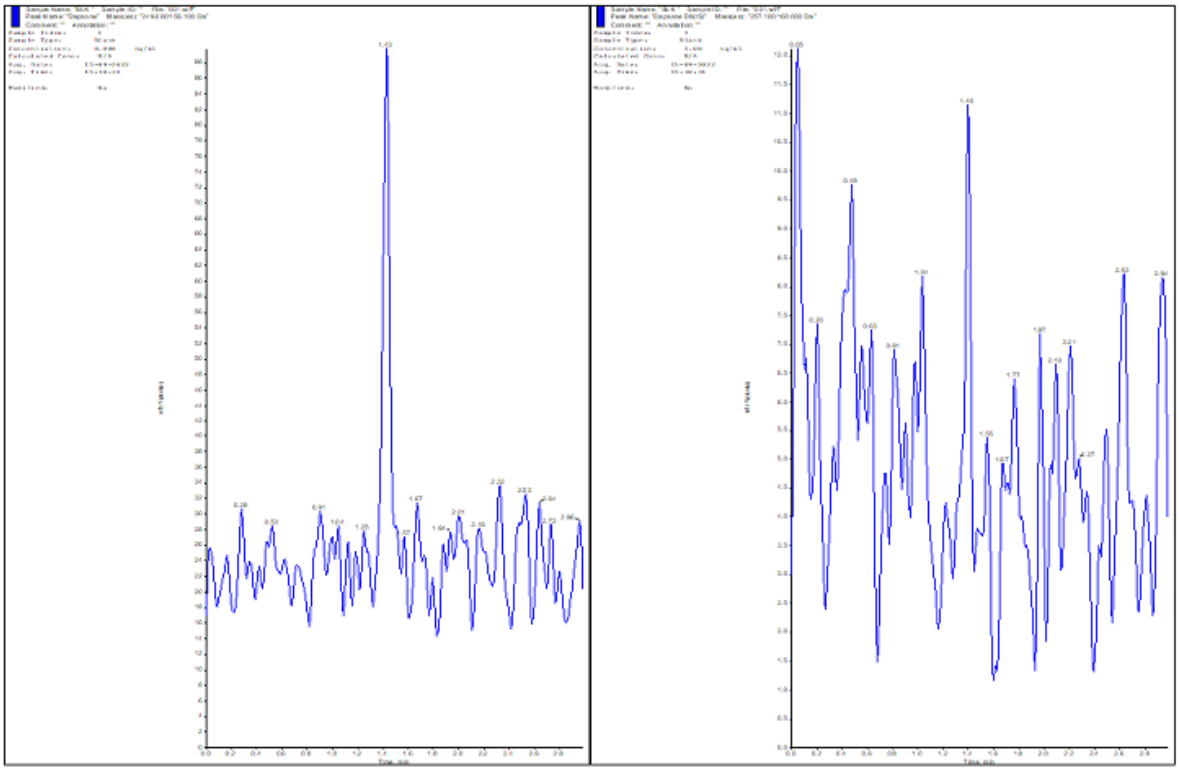
Calibration Curve: Summary of calibration Curve Parameter ≥ 0.9966. Concentration Summary of Calibration Standards Coefficient of variation 0.95 to 4.65%, % Nominal value 91.842 to 105.863%.

Accuracy and precision: Precision and Accuracy-01QC Coefficient of variation 2.28 to 5.78%, QC % Nominal value 98.300 to 109.383%. Precision and Accuracy-02QC Coefficient of variation 2.80 to 5.76%, QC% Nominal value 98.027 to 106.973%. Ruggedness Precision and Accuracy-03 (DA_DC) QC Coefficient of variation 2.82 to 6.32%, QC % Nominal value 97.600 to 107.073%. Precision and Accuracy-04QC Coefficient of variation 2.41 to 5.89%, QC % Nominal value 96.591 to 108.582%.Between-run Precision and Accuracy QC Coefficient of variation 3.28 to 5.49%, QC % Nominal value 97.629 to 108.003%.

Recovery: % Recovery of Analyte LQC 74.217, MQC 75.603, HQC 78.620 and % Recovery of IS75.363. % Recovery of Mean Analyte 76.1467.

Stabilities: STSS of Analyte at LQC and HQC Level at RT Mean % Change is 1.355% for LQC & 0.195% for HQC after 24 Hours 28 Minutes. STSS of IS at LQC & HQC Level at room temperature Mean % Change is 4.398% for LQC and 7.401% for HQC after 24 Hours 29 Minutes. Short Term Working Solution Stability of Analyte at LQC and HQC Level at Room Temperature Mean % Change is 1.233% for LQC and 1.854% for HQC after 23 Hours 55 Minutes. Short Term Working Solution Stability of IS at LQC and HQC Level at Room Temperature Mean % Change is 0.607% for LQC and 0.120% for HQC after 24 Hours 29 Minutes. FT 5th Stability at -70°C ±10°C.

Autosampler Stability at 10°C after 69 Hours 02 Minutes. Bench Top Stability of Analyte in Matrix at Room Temperature after 19 Hours 15 Minutes.



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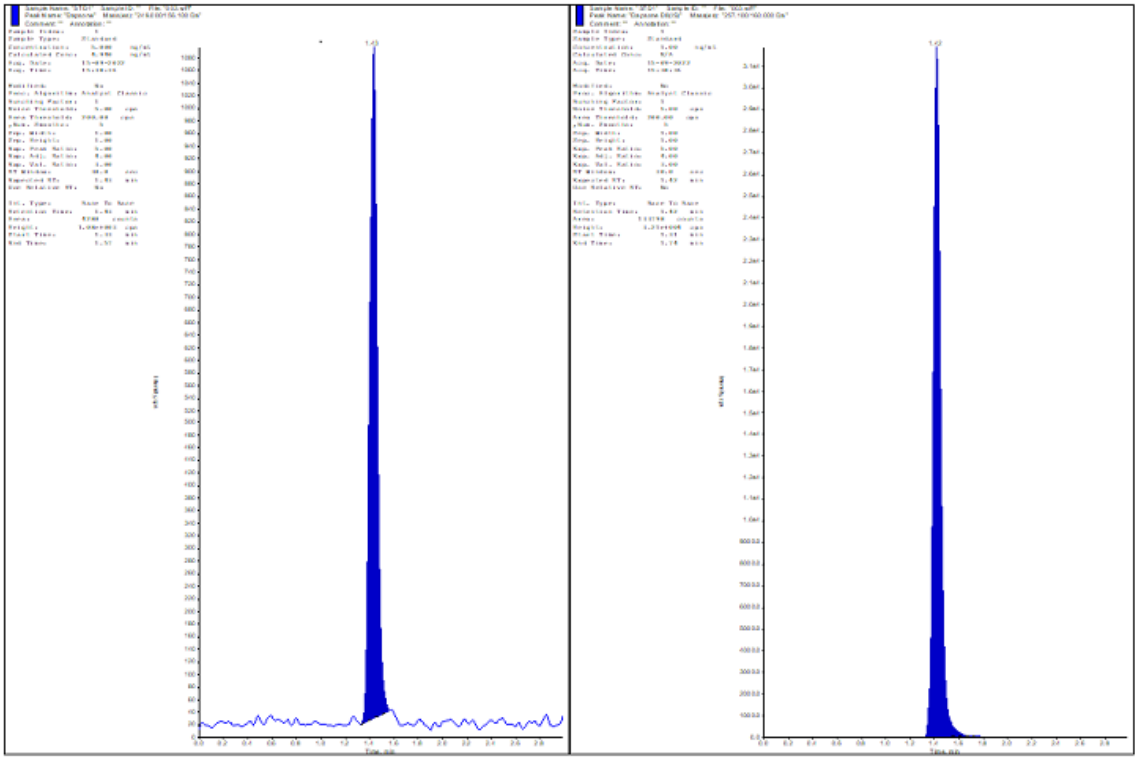

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Figure-3: Chromatograms (I) Blank plasma and (II) LLOQ Dapsone and it IS Dapsone D8.

Table-1: Concentration Summary: Calibration Standards of Dapsone.

Calibration standards	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8
Nominal Conc.(ng/mL)	5.000	10.001	224.740	525.093	1346.392	1923.418	2404.272	3005.340
Range(ng/mL)	4.000	8.501	191.029	446.329	1144.433	1634.905	2043.631	2554.539
	6.000	11.501	258.451	603.857	1548.351	2211.931	2764.913	3456.141
Result Table ID	Conc. Found	Conc. Found	Conc. Found	Conc. Found	Conc. Found	Conc. Found	Conc. Found	Conc. Found
Selectivity	5.054	9.822	206.082	517.403	1342.588	1980.293	2480.956	3144.250
Bulk Spike Check	5.036	9.889	209.322	511.517	1313.318	1988.071	2515.940	3134.453
P & A-1	4.940	10.277	209.086	513.183	1210.804	2027.407	2536.684	3210.652
P & A-2	4.936	10.287	210.810	515.636	1220.038	2019.525	2543.430	3156.053
HE_LE_DI	5.110	9.589	209.510	526.532	1220.418	2001.523	2549.823	3235.533
P & A-3	5.016	9.970	209.634	521.129	1218.915	2035.205	2516.281	3199.929
P & A-4	5.003	10.022	208.020	520.405	1233.109	2044.575	2545.879	3134.090
SOL_STB	5.090	9.669	211.507	526.657	1149.852	2040.973	2571.466	3265.069
EBT	5.013	9.973	213.010	522.817	1219.986	1997.602	2554.793	3153.869
N	9	9	9	9	9	9	9	9
Mean	5.0220	9.9442	209.6646	519.4754	1236.5587	2015.0193	2535.0280	3181.5442
S.D.	0.05936	0.23867	1.99734	5.45553	57.49660	23.83797	26.85130	47.89638
% C.V.	1.18	2.40	0.95	1.05	4.65	1.18	1.06	1.51
% Nominal	100.440	99.432	93.292	98.930	91.842	104.762	105.438	105.863

Table-2: Stability of Dapsone in Matrix.

	% CV		% Stability	
	LQC	HQC	LQC	HQC
Bench Top Stability for 19 Hrs	5.51	2.36	-0.126	4.522
5^{TH}Freeze and Thaw Stability at -70.0°C	4.01	3.77	-3.309	3.248
Auto Sampler Stability for 69 Hrs	3.97	3.36	-1.075	3.799
Long Term Stability at -70.0°C for 54 Days	1.56	2.28	2.520	-1.551
Long Term Stability at -20.0°C for 54 Days	2.71	3.07	-0.259	-0.824

Conclusion

A sensitive, rapid and simple Liquid Chromatography Mass Spectrometric method has been developed and validated for the quantification of Dapsone in human plasma matrix using K_2EDTA as anticoagulant. Sensitivity has been reached at conc. of 5.000ng/mL having good signal to noise ratio for Dapsone. The method can be prosper applied to bioequivalence/ bioavailability subject analysis of Dapsone.

Acknowledgement

The authors are indebted to founder and CEO Mr. Puneet Verma Director of Synergen Bio Pvt. Ltd. The authors gratefully acknowledge Synergen Bio Pvt. Ltd. to carry over out this work to full fill all the requirements.

References

1. Shepard C.C. (1982). Leprosy today. N Engl J Med (307), 640-641.

2. Waters MFR (1983). New approaches to chemotherapy for leprosy. *Drugs*, (26), 465-467.

3. Powell R. D., DeGowin R. L., Eppes R.B., McNamara J. V. & Carson P. E. (1967). The antimalarial and hemolytic properties of 4-4'diaminodiphenylsulfone (DDS). *Int J. Lepr*, (35), 590-604.

4. Rees R.S., Altenbem D.P., Lynch J.P. & King L.E. Jr (1985). Brown recluse spider bites: A comparison of early surgical excisionversus dapsone and delayed surgical excision. *Ann Surg*, (202), 659-663.

5. Swain A.F., Ahmad R.A., Rogers H.J., Leonard J. N. & Fry L. (1983). Pharmacokinetic observations on dapsone in dermatitis herpetiformis. *Br J Dermatol*, (108), 91-98.

6. Kumano K., Tani M. & Murata Y. (1983). Dapsone in the treatment of miliary lupus of the face. *Br J Dermatol*, (109), 57-62.

7. Grindulis K.A. & McConkey B. Rheumatoid (1984). Arthritis: The effects of treatment with dapsone on hemoglobin. J. Rheumatol, (11), 776-778.

8. Poulsen A., Hultberg B., Thomsen K. & Wantzin G. L. (1984). Regression of Kaposi's sarcoma in AIDS after treatment with dapsone. *Lancet*; (1), 560.

9. Yu-Luan Chen, Heiko Junga, Xiangyu Jiang&Weng Naidong (2003). Simultaneous determination of theophylline, tolbutamide, mephenytoin, debrisoquin, and dapsone in human plasma using high-speed gradient liquid chromatography/tandem mass spectrometry on a silica-based monolithic column. *J Sep Sci*., 26(17), 1509–1519.

10. Eljaschewitsch J., Padberg J., Schürmann D. & Ruf B. (1996). Highperformance liquid chromatography determination of pyrimethamine, dapsone, monoacetyldapsone, sulfadoxine, and N-acetyl-sulfadoxine after rapid solid-phase extraction. *Ther Drug Monit*, 18(5), 592-597.

11. Philip, P. A., Roberts, M. S., & Rogers, H. J. (1984). A rapid method for determination of acetylation phenotype using dapsone. *British journal of clinical pharmacology*, 17(4), 465-469.

12. Tigecycline, P. R. O. D. U. C. T., Reviewer, P., Wang, Y., Leader, T. E. A. M., Jarugula, V. R., Leader, P. T., & Gobburu, J. (2018). Clinical pharmacology & biopharmaceutics review.

13. Guideline, I. H. (2022). Bioanalytical method validation and study sample analysis M10. ICH Harmonised Guideline: Geneva, Switzerland.

14. Lin, Y. J., Wang, Y. C., Huang, H. H., Huang, C. H., & Lin, P. L. (2022). Efficacy and safety of remifentanil for endoscopic ultrasound-guided tissue acquisition: a single center retrospective study. *Surgical Endoscopy*, 36(9), 6516-6521.

15. Chae, S. U., Kim, J. S., Lee, C. B., Jo, S. J., Min, K. L., Chang, M. J., & Bae, S. K. (2023). A Sensitive, Simple, and Fast LC–MS/MS Method for Quantification of Remifentanil in Human Plasma: Applications in Critically Ill Patients' Plasma during Extracorporeal Membrane Oxygenation. *Separations*, 10(6), 359.