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Dried blood spots for the quantification simultaneous administration of the antipsychotic and antidepressant drugs

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Abstract: Liquid chromatography-tandem mass (LC MS/MS) was used for the determination of therapeutic drug monitoring (TDM) of the three antipsychotics (aripiprazole, quetiapine, and olanzapine) and three antidepressants (paroxetine, Escitalopram, and sertraline) drugs simultaneously. Both groups of drugs can be concurrently used to treat behavioral disorders. It appears that there is no test for the rapid detection of all six compounds simultaneously using LC MS/M, despite the fact that several analysis publications found these drugs individually. 50µl of taken from finger pricks as dried blood spots (DBS) spiked with sample solution containing the six understudied drugs was extracted. A C_{18}-BEH column with a mobile phase made up of gradient elution ammonium acetate with acetonitrile in methanol. The total run time of this method is about 5.5 min. LC MS/MS showed an excellent linearity in the range of 5-100ng ml^{-1} with a correlation coefficient (r) >0.992. The values of the intra- and inter-day precision of the tested drugs satisfy the regulatory requirements' acceptance criteria. The test was approved in accordance with accepted standards for bioanalytical procedures, and it can be successfully applied for therapeutic drug monitoring studies for the tested drugs if they administered concurrently or individually.

Keywords: Antidepressant, antipsychotic, dried blood spot, liquid chromatography-tandem mass spectrometry and method validation.

INTRODUCTION

Aripiprazole (ARP) quetiapine (QTN) and olanzapine (OLZ) are antipsychotic medications, while paroxetine (PRX), Escitalopram (ESP) and sertraline (SRT) are commonly prescribed antidepressants, both address concomitant behavioral issues (Pfennig *et al*., 2013, Hui *et al*., 2019). However, there is a significant interpatient variation in the way the therapy is responding. Additionally, it is commonly recognized that individuals with psychotic symptoms frequently demonstrate poor compliance with medication therapy (Garcia *et al*., 2016, Jawad *et al*., 2018). This situation can also raise the illness burden, the rate of inpatient admission, the suicide rate, and the expense of treatment (Stephenson *et al*., 2012, Higashi *et al*., 2013).

Additionally, serious adverse effects of these medications have been occurred; include metabolic irregularities, hyperglycemia, extra pyramidal symptoms, cardiovascular problems and irreversible extra pyramidal symptoms (Bobo *et al*., 2013, Bousquet and Purper-Ouakil, 2018). To measure drug concentrations of these medications in the blood and prevent anticipated side effects (extra pyramidal side effects), therapeutic drug monitoring (TDM) appeared to be effective. TDM is additionally acknowledged as having potential benefits for enhancing treatment effectiveness, managing compliance and preventing side effects in people taking antipsychotics.

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Currently, several analytical adherence methods is often done utilizing conventional analysis tests made from venous whole blood, such as plasma or serum (Keefe *et al*., 2007, Hiemke *et al*., 2018). However, venous whole blood sampling must only be done by qualified professionals and can be uncomfortable and anxiety-inducing, especially for the mental population. These drawbacks might be solved by other sampling techniques like micro sampling. Sampling of dried blood spots (DBS) is one of the most often used techniques and was initially published in 1963 (Guthrie and Susi, 1963).

DBS is marketed as a simple, affordable, minimally intrusive and acceptable at-home sample method. DBS was utilized, among other things, for therapeutic drug monitoring (TDM) of antipsychotics (Jacobs *et al*., 2021, Patteet *et al*., 2015, Sharma *et al*., 2014, Meesters and Hooff, 2013, Tron *et al*., 2017, Martial *et al*., 2017). In order to create a less invasive sample technique for TDM in a pediatric population, this form of sampling is therefore particularly intriguing. It is trendy to find ways to determine how many medicines at once that is quick, affordable, expeditious and a quick study of patient adherence and intoxication.

TDM determination in plasma and/or serum of antipsychotic drugs was all concurrently determine by several publications using the LC MS/MS technology (Fisher *et al*., 2013, Wang *et al*., 2015, Proença *et al*., 2020, Qi *et al*., 2021, Martial *et al*., 2017, Zhou *et al*., 2004, de Lima Moreira *et al*., 2022, Flanagan *et al*., 2023).

While, antidepressant drugs were simultaneously measured by authors such as (Wang *et al*., 2015, Déglon *et al*., 2010). Additionally, combination of both antipsychotic and antidepressants drugs were determined through publications (Wang *et al*., 2015, Pronk *et al*., 2023, Kumar *et al*., 2023).

Currently, the DBS method was used recently for the determination of antipsychotics (Patteet *et al*., 2015, Jacobs *et al*., 2021, Stern *et al*., 2020) or use to determined antidepressant drugs (Déglon *et al*., 2010, Barfield and Wheller, 2011, Antunes *et al*., 2016). Additionally, combination of both antipsychotics and antidepressants was determined by DBS (Moretti *et al*., 2019). Despite the fact that several of these medications have been identified simultaneously, as was described above, it appears that no such test exists for the quick detection of all six substances using LC MS/MS. Additionally these published articles (Déglon *et al*., 2010, Stern *et al*., 2020, Barfield and Wheller, 2011, Antunes *et al*., 2016, Moretti *et al*., 2019) also have drawbacks, such as a higher limit of quantification (Déglon *et al*., 2010, Barfield and Wheller, 2011), a large volume of plasma sample to process (Antunes *et al*., 2016), an extended analytical run time, and/or a time-consuming sample clean-up procedure (Moretti *et al*., 2019, Stern *et al*., 2020).

Several analytical measurements should be monitored such as linearity, selectivity and recovery, etc. during the development of quantitative DBS study. Even if regulatory organizations have not yet delivered detailed procedures for DBS method validation, approvals were done by several authors (Bousquet and Purper-Ouakil, 2018, Timmerman *et al*., 2011) who emphasize the importance of doing an extensive analytical validation before considering replacing plasma sample with DBS sampling.

Verification of the DBS assay employing ultra-high-performance liquid chromatography- tandem mass spectrometry LC MS/MS, for the tested drugs, was the aim of this study, which allows for a direct finger-prick sample at the patient's home (DBS) and suitable applications for TDM of Aripiprazole (ARP), quetiapine (QTN) and olanzapine (OLZ) as antipsychotic medications, paroxetine (PRX), Escitalopram (ESP), and sertraline (SRT) as antidepressants drugs all at once.

MATERIALS AND METHODS

Materials

Aripiprazole, Quetiapine, Olanzapine, Paroxetine, Escitalopram and Sertraline were gifts from Saudi Pharmaceutical Industries & Medical Appliances Corporation (Spimaco, Riyadh, Saudi Arabia). Protein Saver Whatman 903 card (Sigma Aldrich, Chemie GmbH, Munich, Germany). All additional chemicals and reagents were of HPLC analytical grade and were used exactly as they were given. With the use of a Milli-Q Reagent Grade water system, water was deionized and purified (Millipore Corporation, Bedford, MX 01730, USA). A regular puncher (Fiskars, Helsinki, Finland) was used for punching the DBS disks out of the spotting cards. Drugfree human whole blood was obtained from volunteers. Blood samples were gently mixed using the HulaMixer sample mixer (Thermo Scientific, Waltham, MA).

Instrumentation

The study utilized a Waters® Acquity HPLC system with a tandem mass spectrometer (triple-stage quadrupole) and electrospray ionization (ESI) source connected to an Acquity binary solvent manager pump and Mass lynx software, version 4.1.

Chromatographic and Mass Spectrometric Conditions

In 45°C, chromatographic separation was accomplished using a UPLC-C_{18}-BEH (Waters^{TM}) Acquity column with 1.7µm particles. 5mM ammonium acetate, 1% acetic acid, 1% acetonitrile, as solvent A (pH 7.0±0.1) made up the mobile phase utilized for the analysis. While solvent B consists of acetonitrile : methanol was 62:38. The overall run time for each sample was 5.5min and the gradient elution profile is shown in table 1. The sample injection volume was 10µl and the autosampler was maintained at 10°C. A triple-quadruple LC/tandem mass spectrometric detection system (Water^{TM}) with Multiple reaction monitoring (MRM) chromatograms in the electrospray ionization (ESI) positive mode was chosen to all of the identified six drugs were found using, with a dwell period of 0.5 seconds.

At the desolvation gas (nitrogen) flow was set as 800 l/h. The condition related to the temperature of the desolvation line, temperature of the source and the nebulizer's temperature were recorded as 500, 150, and 150°C (7 psi), respectively. The collision gas (argon) flow rate was 0.14 ml/min, with a capillary voltage of 2.0 kV and a cone voltage of 3 volt. The determined multiple reaction monitoring (MRM) transitions of the previously indicated conditions drugs were shown in table 2.

Calibration criteria and quality control samples preparation

At a concentration of 0.5 mg/ml, each analyte was generated as a separate stock solution in methanol. In order to make an intermediate mixed solution, these stock solutions were combined and diluted with methanol that contained the six analytes for aripiprazole, quetiapine, olanzapine, paroxetine, escitalopram, and sertraline at concentrations of 5, 10, 20, 40, 60, 80 and 100ng/ml. In a brown bottle, the mixed solution was kept at - 30^{o}C. Every day, working solutions for each calibrator and

quality control (QC) level were made by diluting the mixture solution in methanol. In order to identify errors in solution production, two distinct batches of mixed solutions (one for QC and one for calibrators) were prepared from two batches of stock solutions for each analyte. A working solution was added to 50 l of drug-free human blood in aliquot. For each drug, concentrations in calibrators and QC samples were 5, 10, 20, 40, 60, 80, and 100ng/ml. After 10 minutes of gentle mixing, the tubes were stabilized for 15 minutes before blood was dried on a DBS card. King Saud University's Institutional Review Board Committee (IRB) has given the project ethical permission; Research Project No. E-22-435. It was carried out in conformity with the Helsinki Declaration. Written informed consent was obtained from every study subject.

Sample extraction procedure

The QC and calibrator samples were produced by pipetting 50 l of spiked blood onto Whatman 903 paper and letting it dry for 4 to 24 hours at room temperature. Using a mechanical 6-mm punch, DBS disks were extracted and then pushed into Eppendorf tubes. Between samples of high and low concentrations, the puncher was cleaned with ethanol to prevent carryover, and spots were punched out. In order to create a blank sample, 200 micro liters of the extraction solution were mixed with acetonitrile: Formic acid (90:10) without IS. Samples were sonicated for 10 minutes after being vortexed for one minute, and then had nitrogen evaporated from them. The samples were reconstituted and 1.5 ml of the mobile phase was added to the auto sampler vials. In only 10 µl, the completed extract was fed into the LC-MS/MS apparatus.

Validation procedures

The thorough validation assay was conducted in line with the Food and Drug Administration's and the European Medicines Agency's recommendations for bioanalytical technique validation (Committee for Medicinal Products for Human Use, 2006) (FDA, 2001, Peters *et al*., 2007).

Stability

QCs to assess the stability of the analytes in the DBS samples, DBS samples at low and high concentrations were created and stored under varied conditions. Along a newly created calibration curve, all QCs were checked in triplicate using new QCs. For each analyte, the measured values were compared to the hypothetical value. Bias within 15% of the nominal values was acceptable in order to make the conclusion that the analyte was stable in DBS samples under the investigated cases. The stability of the analytes was evaluated in the following storage conditions: 24 hours, 72 hours, a week, ten days, and a month at room temperature; 1, 3, 7, and 30 days in the refrigerator (4-8°C). To imitate hot or humid conditions over the same days, certain DBS samples were kept at 45°C in an oven.

STATISTICAL ANALYSIS

All statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 10.0. Correlations were considered statistically significant if calculated P values were 0.05 or less.

RESULTS

Optimization of chromatographic condition

In order to lower column pressure and enhance resolution, the column temperature was 45oC. Ten microliters of reconstituted solution were injected for analysis because the UPLC-C18-BEH column (1.7 mm 100 mm, 5 m) only needed a tiny injection volume and allowed for the acquisition of peaks with superior peak shapes. SRT, OLZ, ESP, PRX, QTN, and ARP could be determined using a highly selective approach made possible by the LC MS/MS in the MRM mode. As indicated in table 2, the protonated molecule was located at m/z 305 for SRT, 313 for OLZ, 325 for ESP, 330 for PRX, 384 for QTN, and 448 for ARP. SRT, OLZ, ESP, PRX, QTN, and ARP had retention times of roughly 4.97, 2.6, 4.09, 4.4, 4.21, and 4.56 minutes, respectively. fig. 1, represent an ESI+ mass spectrum (MRM) of tested drugs, while, fig. 2 and 3 represent the individual and cumulative chromatograms of control human plasma, respectively.

Calibration curves

Assay validation

Selectivity and specificity

There were no interferences during the analyte retention times for any of the six dried blood samples. Less than 15% of the regions in spiked samples were present in blank samples at the LLOQ concentration. Therefore, it was concluded that the method was sensitive and selective enough.

Linearity, limit of quantification, limit of detection

Table 3, showed the method's linearity for all analytes (SRT, OLZ, ESP, PRX, QTN and ARP). An optimum fit was made by weighting factor of 1/. The back-calculated concentrations of the calibrators were within the permitted range (15%), and each validation analysis's coefficient of correlation was more than 0.992.

Precision and Accuracy

Table 4 provides results of accuracy and within-day and between-day imprecision measurements made at three concentrations. They met the requirements for approval because the bias and CV for the LLOQ and other QC concentrations were both less than 20% and 15%, respectively.

Table 1: The condition for the gradient elution of the tested drugs of LC MS/MS

Time	Flow (ml/min)	Solvent A %	Solvent B %
0	0.45	95	5
0.7	0.45	95	5
3.5	0.45	5.0	95
4.5	0.45	5.0	95
5	0.45	95	5
5.5	0.45	95	5

Table 2: The setting for the Mass Spectrometry of the tested drugs in the DBS Assay

Compound	Abb.	Formula Mass	MRM parent> daughter (M/Z)	Cone Voltage	Collision Energy	retention Time (minutes) Mean ± SD
Sertraline	SRT	C17H17Cl2N	305.98 > 123.02	2.0	44	4.97± 0.21
			305.98 > 158.92	2.0	28	4.97 ±0.21
Esciltalopram	ESP	C20H21FN2O	325.07 > 108.98	2.0	24	4.09 ±0.26
			325.07 > 262.05	2.0	22	4.09 ±0.26
Parexetine	PRX	C19H20FNO3	330.05 > 70.04	2.0	30	4.40 ±0.18
			330.05 > 192.09	2.0	20	4.40 ±0.18
Aripiprazole	ARP	C23H27Cl2N3O2	448.05>285.11	4.0	28	4.56 ±0.12
			448.05>176.05	4.0	24	4.56 ±0.12
Quetiapine	QTN	C21H25N3O2S	384.07>253.03	6.0	22	4.21 ±0.23
			384.07>221.10	6.0	34	4.21 ±0.23
Olanzapine	OLZ	C17H20N4S	313.17>198.04	2.0	44	2.60 ±0.31
			313.17>256.06	2.0	22	2.60 ±0.31

Table 3: Mass Tandem retention times calibration coefficients for the various tested drug

Drug	Calibration curve (ng/ml)	Correlation coefficients mean ± SD	Linear equation
SRT	5, 10, 20, 40, 60, 80 and 100	0.994 ± 0.002	Y= 12242x + 990
OLZ	5, 10, 20, 40, 60, 80 and 100	0.991 ± 0.002	Y= 979642x + 377
ESP	5, 10, 20, 40, 60, 80 and 100	0.998 ± 0.002	Y= 48106x + 392
PRX	5, 10, 20, 40, 60, 80 and 100	0.996 ± 0.002	Y= 32908x – 520
QTN	5, 10, 20, 40, 60, 80 and 100	0.998 ± 0.002	Y= 45732x + 152
ARP	5, 10, 20, 40, 60, 80 and 100	0.992 ± 0.002	Y= 451958x +284

Table 4: The Accuracy and imprecision for within-day and between-day of the DBS Assay

Analyte	Concentration, ng/ml	Within-Day Imprecision (CV%)	Between-Day Imprecision (CV%)	Within-Day Accuracy (Bias %)	Between-Day Accuracy (Bias %)
SRT	10	13.2	14.4	12.6	14.3
	50	7.3	8.5	6.8	7.2
	100	5.3	4.9	4.8	5.2
OLZ	10	14.3	14.1	13.6	13.2
	50	8.7	8.2	5.9	6.4
	100	4.7	5.3	5.9	3.6
ESP	10	13.1	12.8	12.2	14.2
	50	8.1	6.2	6.7	8.3
	100	3.7	4.6	4.9	5.4
PRX	10	12.6	11.1	9.5	13.2
	50	9.3	7.4	5.4	9.3
	100	5.5	5.1	3.5	6.2
QTN	10	13.2	13.7	14.2	13.2
	50	8.3	7.7	6.7	8.2
	100	4.4	5.7	4.9	3.8
ARP	10	14.2	12.2	13.7	12.9
	50	7.5	6.9	7.6	7.2
	100	3.7	4.7	3.9	3.5

Table 5: The matrix effect (ME) and the extraction recovery (ER) of QC Sample of Analytes in DBS

Analyte	Matrix Effect				Recovery			
	QC Low		QC High		QC Low		QC High	
	Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)	Mean (%)	CV (%)
SRT	102	1.7	102	2.7	94	5.3	93	3.3
OLZ	93	2.3	98	1.9	97	4.7	95	3.8
ESP	99	2.8	94	2.9	102	3.9	103	4.1
PRX	89	3.1	103	3.6	104	5.2	93	4.6
QTN	94	4.6	96	2.6	97	4.1	103	5.3
ARP	94	3.1	101	4.7	93	3.8	96	5.1

Table 6: Stability of the tested drugs in auto sampler and after freezing–thaw cycles in different temperature for 4 weeks (n = 3, Mean ± SD).

Temp/days	SRT Mean % ± SD	OLZ Mean % ± SD	ESP Mean %± SD	PRX Mean %± SD	QTN Mean %± SD	ARP Mean % ± SD
-30°C						
1	88 ± 5.2	95 ± 3.3	99 ± 3.4	103 ± 1.6	112	99
3	95 ± 3.7	98 ± 4.3	113 ± 2.3	90 ± 5.3	86 ± 4.3	111 ± 3.5
7	102 ± 1.9	95 ± 4.7	101 ± 4.3	111 ± 3.3	94 ± 3.7	95 ± 5.7
10	112 ± 1.3	92 ± 6.1	96 ± 5.6	99 ± 4.7	89 ± 6.3	88 ± 6.6
30	104 ± 2.7	101 ± 1.9	94 ± 4.3	95 ± 5.1	92 ± 7.1	89 ± 6.8
4°C						
1	104 ± 3.1	95 ± 3.7	99 ± 3.1	111 ± 1.6	95 ± 5.3	90 ± 6.5
3	112 ± 0.9	98 ± 1.3	105 ± 2.4	95 ± 4.3	101 ± 1.5	95 ± 4.1
7	104 ± 1.4	94 ± 2.8	108 ± 2.7	98 ± 3.6	114 ± 0.9	99 ± 4.3
10	98 ± 2.6	112 ± 3.4	113 ± 2.4	92 ± 6.3	102 ± 1.5	108 ± 2.8
30	94 ± 3.4	104 ± 1.8	92 ± 6.3	102 ± 4.3	94 ± 4.4	101 ± 3.7
25°C						
1	95 ± 4.5	108 ± 2.8	95 ± 4.3	105 ± 2.2	95 ± 4.9	107 ± 4.2
3	99 ± 2.1	113 ± 1.1	103 ± 5.2	103 ± 3.7	104 ± 5.3	112 ± 1.2
7	108 ± 1.1	92 ± 3.4	114 ± 1.6	93 ± 5.8	107 ± 2.2	105 ± 3.5
10	101 ± 2.8	95 ± 4.7	105 ± 3.3	98 ± 4.1	112 ± 1.1	97 ± 3.7
30	111 ± 1.8	90 ± 6.3	109 ± 2.5	90 ± 6.5	105 ± 3.5	91 ± 6.2
45°C						
1	104 ± 3.2	99 ± 1.2	95± 1.2	114 ± 1.1	97 ± 6.2	106 ± 5.4
3	111 ± 1.5	94 ± 5.5	99 ± 4.5	105 ± 3.3	108 ± 5.4	109 ± 3.6
7	98 ± 4.3	107 ± 3.3	108 ± 3.3	95 ± 4.7	113 ± 3.2	112 ± 1.3
10	95 ± 4.8	111 ± 1.8	101 ± 4.4	93 ± 6.2	92 ± 6.1	96 ± 4.8
30	91 ± 5.6	113 ± 2.3	90 ± 5.3	105 ± 4.2	102 ± 4.3	93 ± 3.3

Carryover

No carryover was seen in the validation trials for any analytes since the responses of the blank sample following injection of a ULOQ sample were less than 20% of the responses of the LLOQ samples.

Extraction recovery (ER)

The recoveries and matrix effects presented from six tested drugs were shown in Table 5. Both low and high values for all analytes recovery rates mean % were approximately around 100%, while the coefficients of variation of the tested drugs (CV%) were less than 15%, the enhancement remained steady across samples from various sources without change.

Stability studies

The stability of the tested drugs (SRT, OLZ, ESP, PRX, QTN and ARP) were tested in four temperature conditions (-30, 4, 25 and at 45°C) in different time intervals 1, 3, 7, 10 and one month. According to data represented in table 6, all the tested drugs remained stable during storage conditions up for 30 days at all tested temperatures investigated (-30, 4, 25 and 45°C), since the loss percentage is less than 15% in all calculated data (table 6).

DISCUSSION

According to the standards of the European Medicines Agency and the Food and Drug Administration, the

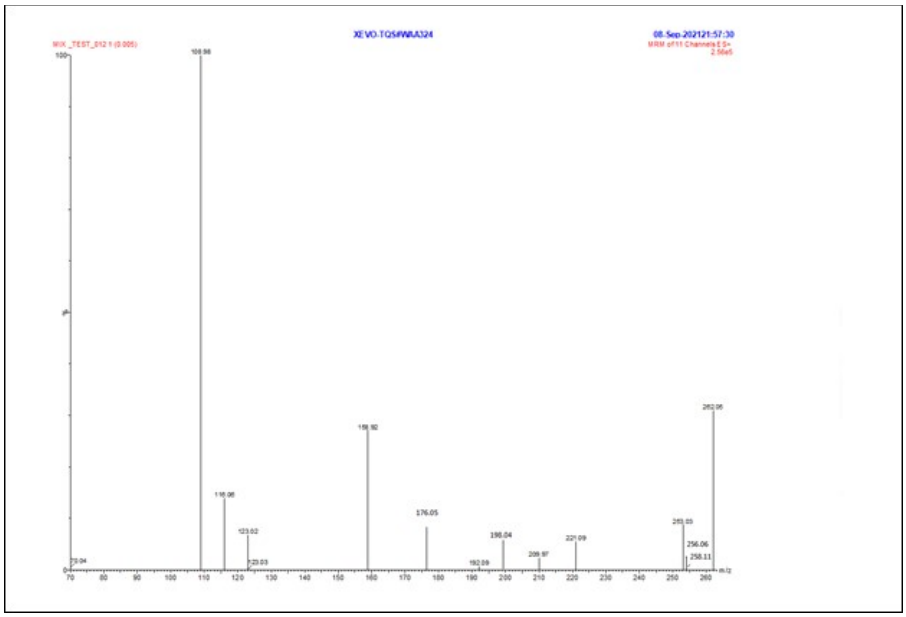

Fig. 1: Product ion spectra of [M+ H]+ of Aripiprazole, Quetiapine, Olanzapine, Paroxetine, Escitalopram and Sertraline fragmentation ion scans. Y-axis is Relative intensity (cps); X-axis is mass-to-charge (m/z, Da).

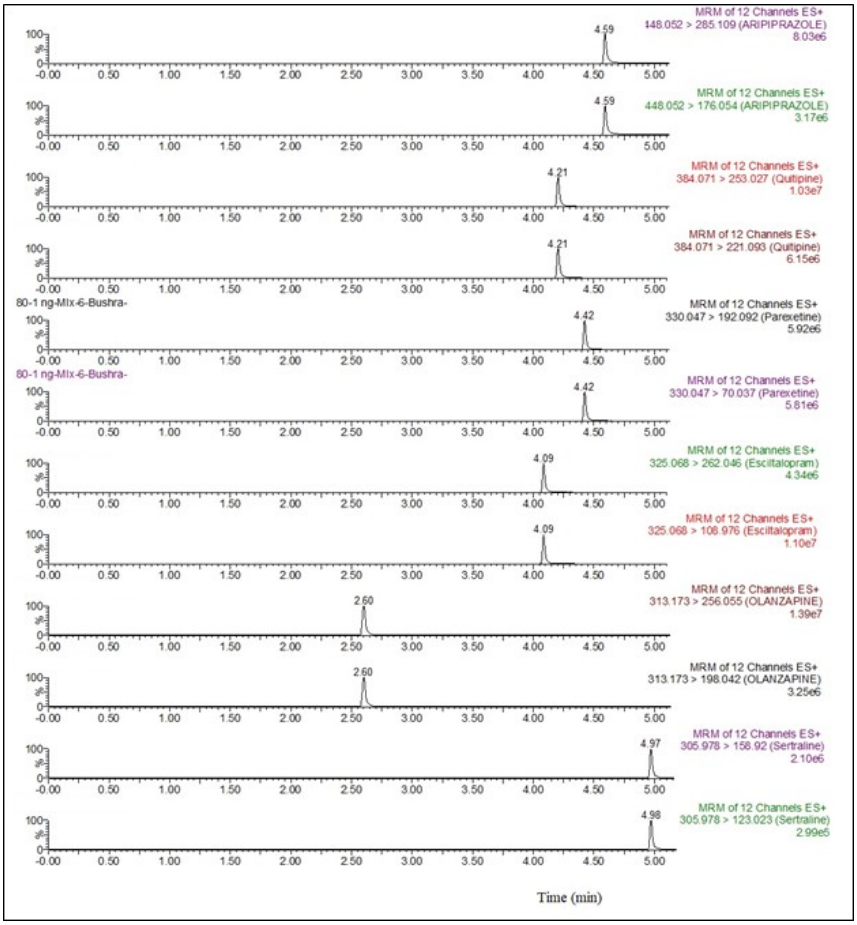

Fig. 2: Individual Chromatograms of standard tested drugs as 1ng/ml) in control dried blood samples (Aripiprazole, Quetiapine, Olanzapine, Paroxetine, Escitalopram and Sertraline) using UPLC-C_{18}-BEH column (1.7 mm × 100 mm, 5 µm). Y-axis is Relative intensity (cps); X-time (min).

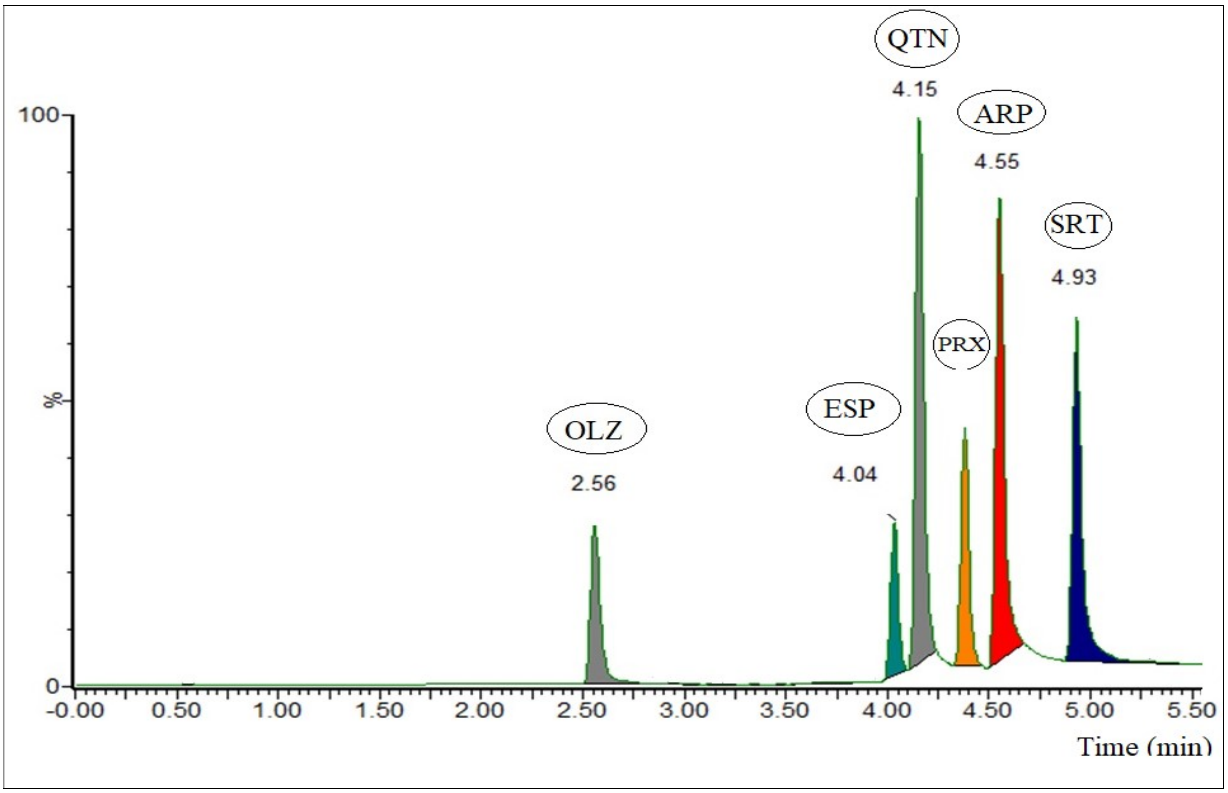

Fig. 3: Cumulative Chromatograms of standard in control dried blood samples. 5 ng/ml of tested standard (Aripiprazole, Quetiapine, Olanzapine, Paroxetine, Escitalopram and Sertraline) using UPLC-C_{18}-BEH column (1.7 mm × 100mm, 5µm). Y-axis is Relative intensity (cps); X-time (min).

analytical method for the simultaneous measurement of three antipsychotics (ARP, QTN, OLZ) and three antidepressants (PRX, ESP, SRT) in DBS in DBS was thoroughly validated. Every analyst satisfies the acceptance standards for accuracy and repeatability.

Liquid chromatography combined with electrospray ionization tandem mass spectrometry (LC-MS/MS) is used to quantitatively identify tested substances in biological matrices such dried blood spots) is increasingly frequently used (DBS). With results that corresponded with FDA bioanalytical requirements, the developed LC-MS/MS approach provided accurate simultaneous estimation of six tested medications (SRT, OLZ, ESP, PRX, QTN and ARP) in DBS. A simple one-step preparation procedure that can quantify up to 5ng/ml of the tested substances with a rapid extraction time of 5.5 minutes. According to the study's findings, DBS home sampling for TDM is associated with lower healthcare and patient expenses as well as greater patient confidence.

Although internal slander was not added to the sample mixture in this investigation, such quantifications of the tested six mixture medications were more reliable and comparable among themselves. In terms of accuracy and recovery, the findings were sufficient for control comparison.

High recoveries and minimal matrix effects were demonstrated by an efficient and straightforward extraction procedure of the resultant drug combination. It's interesting to note that the extraction technique wasn't developed using any internal slandered (IS). In fact, it was discovered that the method's performance was enough without the use of IS because each analytic medication was measured in accordance with the other drugs employed in the mixture.

All studies of drug mixtures demonstrated repeatability and accuracy that met the standards for acceptance. All between-day and within-day accuracy and recoveries fell below 14.4%, which is just shy of the allowable threshold (15%). Additionally, even at low concentrations of 10ng/ml, all analytes shown acceptability for the repeatability and accuracy measurements.

As the study previously stated, several authors have published DBS for a combination of antipsychotic and/or antidepressants (Patteet *et al*., 2015; Jacobs *et al*., 2021; Stern *et al*., 2020; Déglon *et al*., 2010, Barfield and Wheller, 2011, Antunes *et al*., 2016, Moretti *et al*., 2019); however, none of the articles have included the six mixtures of drugs used.

At the four examined temperatures, all analytes mixes were discovered to be stable in DBS for at least one month (-30, 4, 25 and 45°C). This is relevant for that the filter paper contained the DBS samples can withstand the different temperature even hot climate during transport, shipment and handling process. As a result, it appears that examination of DBS samples sent by ordinary mail in such chilly or hot weather conditions is acceptable.

CONCLUSION

A rapid, delicate, and simpler technique has been developed and validated utilizing LC-MS/MS technology for DBS quantification of three antipsychotics (ARP, QTN, OLZ) and three antidepressants (PRX, ESP, SRT). This method has a shorter run time of 5.5 min. Despite the use of DBS, sensitivity and regeneration were maintained and were adequate to determine the patient's MET. The method showed good linearity for all tested drugs in the range of 5-100 ng/ml. This method successfully met each requirement for validation set forth by the EMA and FDA forms. This method may also be known as DBS home sampling for the TDM of patients receiving six drugs antipsychotic and/or antidepressant used for the treatment of behavior disorder.

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