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RESEARCH ARTICLE

AN ASSESSMENT OF PHTHALATES IN COSMETIC PRODUCTS BY DISPERSIVE LIQUID-LIQUID EXTRACTION METHOD USING HPLC AND LC-MS/MS

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Abstract

A Dispersive liquid-liquid microextraction (DLLME) based analytical method was developed and validated using HPLC-PDA and LC-MS/MS for quantitative determination of phthalates (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Benzyl Butyl Phthalate (BzBP), Dibutylphthalate (DBP), Diethylhexyl phthalate (DEHP), Di-n-octyl phthalate (DOP) in different cosmetic products (After Shave Lotion, Deodorants, Perfume and Liquid Body Lotion). The DLLME based developed and validated analytical method was found specific, sensitive, accurate and precise. The accuracy (% recovery) of the method at a spiking level of 0.1, 0.5 and 1.0 mg L⁻¹ in the different cosmetic product was found in the range of 92-108. The interday and intraday precision (%RSD) of the method was found less than 15. Out of six analyzed phthalates, only four phthalates were detected in different cosmetic products. Dimethyl phthalate (DMP) and Di-n-octyl phthalate (DOP) were detected in aftershave lotion. Diethyl phthalate (DEP) was detected in deodorants. Dibutyl phthalate (DBP) and Diethyl phthalate (DEP) were detected in perfumes. None of the phthalates were detected in liquid body lotion.

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Introduction:-

Phthalic acid esters (Phthalates) are anthropogenic originated bouquets chemical that is widely used as plasticizers to increase the flexibility and durability of the plastic materials. They are widely used in everyday consumer products such as pharmaceutical tablets and capsules, vitamins, adhesives and glues, detergents and surfactants, fishing lures, children's toys, paints, and printing inks, food product containers, textiles, and household items such as shower curtains, vinyl upholsteries, and floor tiles [1, 2].

Phthalates may migrate/volatilized in the outdoor and indoor environment and may found in human urine, breast milk, and blood and baby womb [3, 4]. They are easily leached into the environment leading to exposure of human and animals [5]. Chemically phthalic esters are classified into two groups (i) low molecular weight PEs (ester side chain, one to four carbons), which are highly polar, and used in a variety of personal-hygiene and cosmetic products,

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detergents and fragrances as scent stabilizers or carriers, and (ii) high molecular weight PEs (ester side-chain, five or more carbons), which are used in plastic tubing, food packaging, processing materials and containers[6, 7].

In the cosmetic industry, phthalates are used at large level every day. Cosmetics products are used largely in today's fashion industry to enhance the beauty or to modify to some extent physical appearance. There are several examples of the use of cosmetics across history [8]. Egyptians used scented oils and ointments to clean and soften their skin and mask their body odor. Claudius Galen, an ancient Greek physician, invented cold cream in the second century. Romans used oil-based perfumes. In the seventeenth century in Europe dandy gentlemen used cosmetics in abundance, often to hide that they usually did not bathe themselves. European ladies of the eighteenth-century used to whiten their face with lead carbonate (and lots of them died because of poisoning with lead). During the nineteenth-century, chemicals were used to replace more expensive natural ingredients making cosmetics even more widely used. Commercial use of phthalates in the cosmetic formulation is to reduce the brittleness and cracking of nail polishes and sealants and work as anti-foaming agents in aerosols, and as solvents[9]. (H. J. Koo and B. M. Lee, Estimated exposure to phthalates in cosmetics and risk assessment,[10]. For the extraction of these phthalates from liquid phase sample has attempted various preconcentration technique such as liquid-liquid extraction and solid phase microextraction. These methods are very expensive time consuming and using a huge amount of toxic solvent [11, 12].

Dispersive liquid-liquid phase microextraction is ternary solvent extraction system, In this method, the mixture of low density/high-density solvent (extraction solvent) relative to water and dispersive solvent (water-miscible solvent) is rapidly injected by a syringe into an aqueous sample [13, 14]. A cloudy solution consisting of a very fine droplet of extraction into aqueous phase is formed these emulsified droplets have a great interstitial area and, consequently, the equilibrium is reached rapidly and the extraction is almost instantaneous resulting high preconcentration obtain[15, 16]. The advantages of this pretreatment technique are rapidity, simplicity, low cost, low solvent use, short extraction time, and high recovery and enrichment factors[17]. In the presented work, A DLLME based analytical method was developed and validated using HPLC-PDA/LC-MS for quantitative determination of phthalates in different cosmetic products [18, 19].

Material and Methods:-

Analytical standards and working solutions:

Phthalate standards (Dimethyl phthalate (DMP), Diethyl phthalate (DEP), Benzyl Butyl Phthalate (BzBP), Dibutyl phthalate(DBP), Diethylhexyl phthalate(DEHP), Di-n-octyl phthalate (DOP)) were purchased from Supelco Sigma–Aldrich USA, Fluka Sigma–Aldrich Schweiz, and Rankem Pvt. Ltd. New Delhi, India with 95% or higher purity. All solvents like n-hexane, acetone, Chloroform, and acetonitrile were purchased from Sigma–Aldrich Co., USA, Spectrochem Pvt. Ltd. India and were glass distilled before use. The individual stock solution of the phthalate standard was prepared at approx 500 mg L⁻¹. The intermediate mixture solution of the phthalate standard prepared at approx. 10 mg L⁻¹ by dilution from individuals stock solution. Calibration solution was prepared at 0.05, 0.1, 0.25, 0.5, 1.0, 2.0 mg L⁻¹ serial dilution of Intermediate mixture solution.

Sampling:

20 sample of each like, After Shave Lotion, Deodorants, Perfume, and Liquid Body Lotion was purchase from Location different cosmetic product shop of Lucknow city India. Each Sample was analyzed in triplicate using developed and validated analytical method.

Extraction of Phthalates from a different cosmetic product:

Phthalates were extracted by a dispersive liquid-liquid microextraction method[20, 21] 1 ml of a sample of aftershave lotion, deodorant, perfume, and liquid body lotion seprely inject in 5 ml of dispersive media double distilled water (acidified with 0.2 ml of 0.1% Formic Acid) in 15 ml conical bottom centrifuge tube (Phthalates free). A mixture of sample and dispersive media was a vortex for 2 min. 2 ml disperser solvent acetonitrile and 0.5 ml of extraction solvent chloroform was injected rapidly in a sample using a syringe. Then the sample was sonicated for 5 min at 50 Hz followed by centrifugation at 4000 rpm for 5 min. After centrifugation, the extract of phthalates in chloroform was settled at the bottom of the tube. The extract was taken out carefully using micropipette and transfer to HPLC glass vial. 20 µL of the extract was taken for further analysis.

Analysis:-**HPLC-DAD:**

HPLC (Thermo Scientific Dionex Ultimate 3000) system was equipped with a diode array detector, temperature control module and on line degasser system. The analysis was performed on Thermo Scientific, Acclaim-C18 column (250 mm X 4.5 mm I.D., 5 μ m particle size) at 25^oC using mobile phase of acetonitrile and water at a flow-rate of 1.0 ml/min. The gradient program was as; 80:20 v/v (acetonitrile and water) for 3.0 min, then only acetonitrile was flows at flow rate of 2 ml/min and hold for 9 min, then again flow rate was maintained to 1 ml for 80:20 v/v (acetonitrile and water) and finally hold for 2.1 min. Phthalates were detected at 230 nm and the sample injection volume was 20 μ l.

LC-MS/MS:

Mass spectrometry (MS) detection was carried out by anAcquity UPLC-AB SCIEX API 4000 triple quadrupole mass spectrometer with electrospray ionization (ESI) performed in positive mode. Full-scan spectra (Q1MS) were recorded from m/z 100–500, at a scan time of 0.5 s and an interscan delay of 0.1 s. The set of parameters used is shown as follows: ion source (IS) 28 spray voltage 5500 eV, source temperature (TEM) 400.0 $^{\circ}$ C; atomization gas pressure (GS1)-45 Psi (nitrogen); heated auxiliary gas (GS2)-40 Psi (nitrogen); curtain gas pressure (CUR)- 10 Psi (nitrogen); collision flow (CAD)-4 Psi. The columns selected for this study were Acclaim-C18 column (250 mm X 4.5 mm I.D., 5 μ m particle size). The mobile phaseswereacetonitrile and water. The gradient program was as; 80:20 v/v (acetonitrile and water) for 3.0 min, then only acetonitrile was flows at flow rate of 2 ml/min and hold for 9 min, then again flow rate was maintained to 1 ml for 80:20 v/v (acetonitrile and water) and finally hold for 2.1 min. The molecular ion m/z was identified as Selected-ion monitoring (SIM) of the most abundant ion was used for quantification. The detector of the mass spectrometer was tuned for the maximum sensitivity of the molecular ion (m/z).

Results And Discussion:-

The analytical method was validated with the parameters like; Specificity, the limit of detection (LOQ), the limit of quantification (LOQ), Linearity, Accuracy (% recovery) and precision (% RSD) for determination of phthalate indifferent cosmetic products. Phthalates (Dimethyl Phthalate (DMP), Diethyl Phthalate (DEP), Dibutyl Phthalate (DBP), Diethylhexyl Phthalate (DEHP), Benzyl Butyl Phthalate (BzBP) and Di-N-Octyl Phthalate (DOP)) were extracted from different cosmetic product (After Shave Lotion, Deodorants, Perfume, and Liquid Body Lotion) by dispersive liquid-liquid microextraction method.

The method was found specific and no interference was found at the retention time of analyteswhen solvent blank, matrix blank was analyzed with respect to LOQ level standard (0.1 mg L⁻¹). The limit of detection (LOD) and limit of quantification (LOQ) was found in the range of 0.029 to 0.043 mg L⁻¹ and 0.102 to 0.142 mg L⁻¹. The linear regression analysis of detector response shown that the value of correlation coefficient (R²) was found more than to 0.99 for all kind of phthalate analyzed. The mean accuracy (% recovery) \pm %RSD of the method in different cosmetic product (After Shave Lotion, Deodorants, Perfume, and Liquid Body Lotion) at spiking level of 0.1 mg L⁻¹ was found 108 \pm 5.11 for DMP, 102 \pm 8.79 for DEP, 97 \pm 6.35 for DBP, 107 \pm 8.56 for DEHP, 106 \pm 5.46 for BzBP, 103 \pm 7.86 for DOP. The mean accuracy (% recovery) \pm %RSD at spiking level of 0.5 mg L⁻¹ was found 101 \pm 4.65 for DMP, 98 \pm 7.56 for DEP, 102 \pm 5.89 for DBP, 96 \pm 9.60 for DEHP, 99 \pm 6.15 for BzBP, 96 \pm 5.29 for DOP. Similarly at a spiking level of 1.0 mg L⁻¹ was found 96 \pm 6.06 for DMP, 100 \pm 8.71 for DEP, 101 \pm 6.99 for DBP, 94 \pm 10.26 for DEHP, 90 \pm 6.69 for BzBP, 92 \pm 5.89 for DOP. The interday and intraday precision (%RSD)of the method was found less than 9 and 15 respectively.

The result of method validation parameters shown that the method was specific, accurate and precise for quantitative determination of Dimethyl Phthalate (DMP), Diethyl Phthalate (DEP), Dibutyl Phthalate (DBP), Diethylhexyl Phthalate (DEHP), Benzylbutyl Phthalate (BzBP) and Di-N-Octyl Phthalate (DOP) in After Shave Lotion, Deodorants, Perfume, and Liquid Body Lotion.

The result of analysis of the phthalates in After Shave Lotion, Deodorants, Perfume, and Liquid Body Lotion shows that 0.323 mg L⁻¹ and 0.563 mg L⁻¹ of Dimethyl phthalate (DMP) and Di-n-octyl phthalate (DOP) respectively, were detected in five samples aftershave lotion. In deodorants, 0.475 mg L⁻¹ of Diethyl phthalate (DEP) was detected in 4 samples. In perfume Dimethyl phthalate (DMP) and Dibutyl phthalate (DBP) was detected with the concentration of

0.569 mg L⁻¹ and 423.3 mg L⁻¹ in eight samples respectively. None of the phthalates were detected in liquid body lotion.

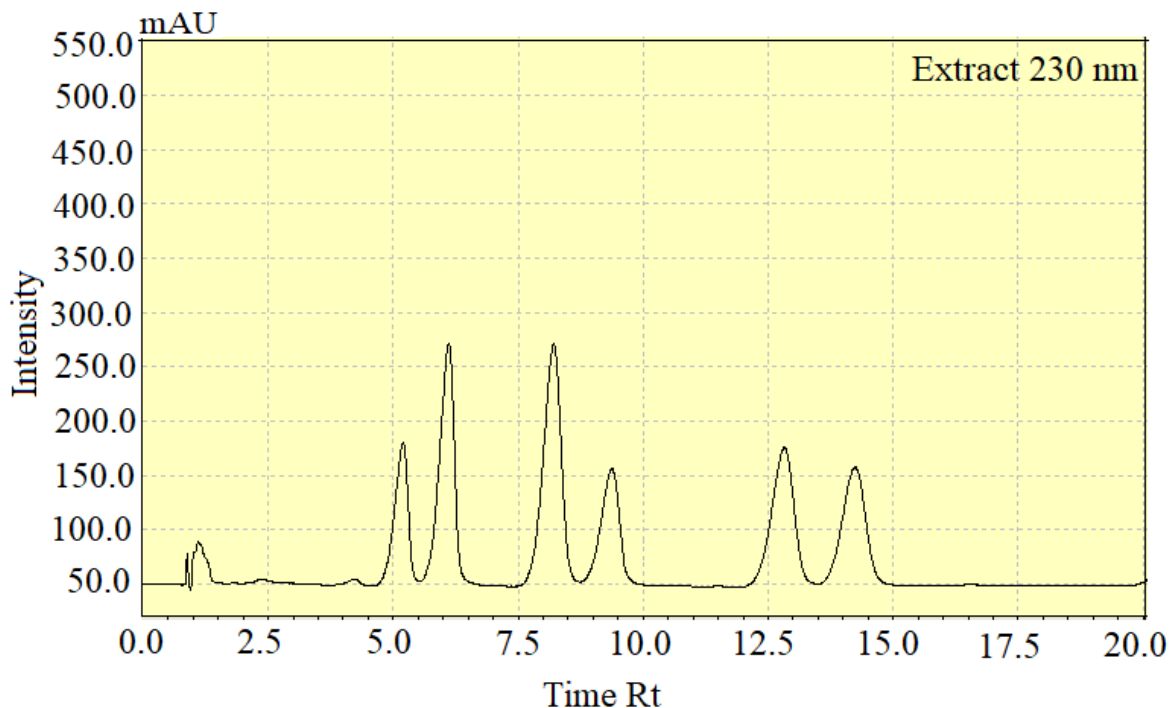


Figure1:- representative chromatogram of phthalates at 0.5 mg L⁻¹ on HPLC.

Table1:- Results of method validation parameters (R², LOD, LOD and precision) and mean % recovery and their standard deviation (SD) in different cosmetic products matrices (After Shave Lotion, Deodrants, Perfume and Liquid Body Lotion).

S.No	Phthalate	RT (min)	R	LOD mg L ⁻¹	LOQ mg L ⁻¹	Mean % recovery and their aSD in different matrices (After Shave Lotion, Deodrants, Perfume and Liquid Body Lotion) at different spiking level			Precision of method in % ^b RSD (n=6)	
						0.1 mg L ⁻¹ (n=7)	0.5 mg L ⁻¹ (n=7)	1.0 mg L ⁻¹ (n=7)	Interday	Intraday
1	Dimethyl phthalate (DMP)	5.32	0.995	0.043	0.142	108±5.11	101±4.65	96±6.06	7	10
2	Diethyl phthalate (DEP)	6.13	0.993	0.039	0.129	102±8.79	98±7.56	100±8.71	9	9
3	Benzylbutyl phthalate (BzBP)	8.36	0.994	0.033	0.109	97±6.35	102±5.89	101±6.99	6	12
4	Dibutyl phthalate(DBP)	9.47	0.998	0.038	0.125	107±8.56	96±9.60	94±10.26	9	13
5	Diethylhexyl phthalate(DEHP)	12.94	0.994	0.029	0.096	106±5.46	99±6.15	90±6.69	7	14
6	Di-n-octyl phthalate (DOP)	14.39	0.992	0.031	0.102	103±7.86	96±5.29	92±5.89	6	13

Note- SD-Standard Deviation, RSD-Relative Standard Deviation, LOD-Limit of Detection, LOQ- Limit of Quantification

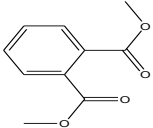
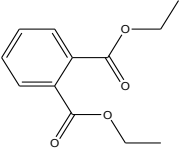
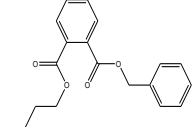
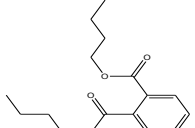
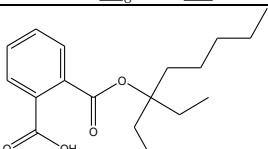
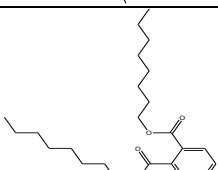
Table2:- Determination of phthalates in four different cosmetic product samples by HPLC.

S. No	Phthalates	Mean level of phthalates (n=20) Detected in different cosmetic
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		product (mg L ⁻¹)			
		After Shave Lotion	Deodorants	Perfume	Liquid Body Lotion
1	Dimethyl phthalate (DMP)	0.323	ND	ND	ND
2	Diethyl phthalate (DEP)	ND	0.475	0.569	ND
3	Benzylbutyl phthalate (BzBP)	ND	ND	ND	ND
4	Dibutyl phthalate (DBP)	ND	ND	0.423	ND
5	Diethylhexyl phthalate (DEHP)	ND	ND	ND	ND
6	Di-n-octyl phthalate (DOP)	0.563	ND	ND	ND

Note- BDL-Below Detection Limit

Table-3:- detail description of phthalates with their parent and product ion.

S.No	Chemical Name	Structure	Molecular Weight	Parent Ion (m/z)	Product Ion
1	Dimethyl phthalate (DMP)		C ₁₀ H ₁₀ O ₄ = 194.1	191	163,77,135,133
2	Diethyl phthalate (DEP)		C ₁₂ H ₁₄ O ₄ = 222.4	223	149,121,93,65
3	Benzylbutyl phthalate (BzBP)		C ₁₉ H ₂₀ O ₄ = 312.3	313	149,91,65
4	Dibutyl phthalate (DBP)		C ₁₆ H ₂₂ O ₄ = 278.3	279	149,93,121,65
5	Diethylhexyl phthalate (DEHP)		C ₂₄ H ₃₈ O ₄ = 390.5	391	149,121,93
6	Di-n-octyl phthalate (DOP)		C ₂₄ H ₃₈ O ₄ = 390.5	391	261,149,65

Conclusion:-

The analytical method developed and validated was accurate and precise and found suitable for the intended purpose of phthalate analysis in a different cosmetic product. The phthalate analysis shows that Dimethyl phthalate (DMP) and Di-n-octyl phthalate (DOP) were detected in aftershave lotion. Diethyl phthalate (DEP) was detected in deodorants. Dibutyl phthalate (DBP) and Diethyl phthalate (DEP) were detected in perfumes. None of the phthalates were detected in liquid body lotion.

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