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Aluminum; HPLC; Differential Refractometer; Antiperspirant Lotion

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# A New Method for Quantifying Aluminum in Antiperspirant Lotion Using a Differential Refractometer and an HPLC Column Containing a Maleate Functional Group

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## Abstract

Aluminum chlorohydrate is a chemical substance employed as an agent to reduce perspiration. As an active antiperspirant agent, it is present in over-the-counter hygiene products at a concentration of up to 25%. The concentration of aluminum chlorohydrate will be estimated indirectly by measuring the concentration of free aluminum ions. Various methodological approaches, including atomic absorption spectroscopy, ion chromatography, and titration, can be employed for the estimation of aluminum. In this study, a novel reverse-phase isocratic liquid chromatography approach was created. The method utilized a differential refractometer, a column packed with a maleate group, and an eluent consisting of 2.0% v/v formic acid. The suggested methodology demonstrates a high degree of precision in quantifying the presence of aluminum in Aldry lotion. According to studies testing accuracy, linearity, and precision, the disclosed analytical technique for quantifying aluminum has a range of 0.125 mg/mL to 0.375 mg/mL. The proposed methodology has a significantly reduced execution time of 10 minutes and is characterized by its cost-effectiveness.

## Introduction

Aldry Lotion is an efficacious anti-perspirant treatment that effectively regulates hyperhidrosis. Utilizing aluminum chlorohydrate as the primary component. The intervention demonstrates efficacy in mitigating hyperhidrosis and additionally serves as a means of regulating perspiration levels. Additionally, it is advantageous for the maintenance of optimal skin health [1]. Aluminum levels can be determined using many analytical techniques, such as Flame Atomic Absorption Spectroscopy (FAAS) [2,3], ion chromatography [4,5] and titration [6,7]. In the back-titration approach, zinc sulfate or zinc acetate will be employed as a titrant for the purpose of determining the presence of aluminum. Due to the reliance on manual titration, there is a potential for variability in the determination of the end point among different individuals. Moreover, the measurement of tiny quantities of aluminum poses challenges when employing titration analytical techniques [8]. The root cause of all ion chromatography issues can be attributed to the quality of water employed in the preparation of samples, standards, and eluents. When using aqueous eluents, the presence of Total Organic Carbon (TOC) in water leads to its accumulation on the surfaces of all polymeric columns. The presence of a high concentration of TOC leads to undesirable effects on the conductivity and UV baselines, causing instability. Additionally, it negatively impacts the efficiency of the column, resulting in broader peak shapes. Furthermore, the column's capacity is compromised, leading to shortened retention times. In certain instances, the TOC can function as a possible site for ion exchange [9]. The aforementioned issues will not manifest themselves in the context of high-performance liquid chromatography. In the context of aluminum determination using ion chromatography, some methodologies employ column derivatization coupled with a fluorescence detector operating at a wavelength of 512 nm. Both the sample and standard preparation techniques require additional time [10]. The utilization of the FAAS technique incurs significant costs [3]. In order to tackle this matter, it is imperative to create analytical methods that are both sensitive and capable of providing high-resolution results for the identification and quantification of aluminum content in anti-perspirant lotions.

This study employed an isocratic HPLC method in conjunction with a Differential Refractometer (DRI, or Refractive Index Detector, RID) to quantitatively determine the levels of aluminum in Aldry lotion. A column packed with a maleate functional group was employed for the purpose of chromatographic separations. The duration of the chromatographic run for each analysis of the samples was 10 minutes. In relation to sensitivity, the HPLC method being presented exhibits comparable performance to that of ion chromatography. A study on forced deterioration was conducted in order to establish the stability-indicating properties of the approach under investigation. The validation of this approach was conducted in accordance with the requirements set forth by the International Convention on Harmonization [11,12]. To date, there is a dearth of literature documenting the analysis of aluminum concentrations in medicinal products utilizing HPLC coupled with a RI detector.

## Materials and Methods

### Chemicals and reagents

For the purpose of this development study, Aldry lotion, manufactured by SunBeam Lifesciences Pvt. Ltd. With batch number SAD2301 and an expiry date of 12/2024, was procured from a pharmacy in India. The following chemicals were procured from Supelco, India: hydrochloric acid fuming, 37% (emprove grade, catalogue no. 100314), 1,2-propanediol (emplura grade, catalogue no. 822324), and urea (ACS grade, catalogue no. 108487). Formic acid with a purity greater than 99% (LC-MS grade, CAS no. 64-18-6) and aluminum chloride (reagent grade, Batch no.N096M21, purity: 99.28%) were acquired from



Avantor, a reputable supplier based in India. PPG-15 stearyl ether (CAS No. 25231-21-4) was obtained from Thirathraj Consolidated Company, located in India. The procurement of cetearyl alcohol (CAS no. 67762-27-0) was carried out through Combi-blocks, Inc., California. The substances Glycerol Stearate and PEG-100 Stearate were obtained from Matangi Industries, located in India. Laureth-23 (CAS No. 9002-92-0) was obtained from Ethox Chemicals, located in the USA. The procurement of Dimeticone (catalogue no. D2200000) was carried out from Sigma-Aldrich, India. Sodium lauryl sulfate (PHR1949) and disodium EDTA (4010-OP) were obtained from Merck, India. The DMDM Hydantoin compound (CAS No. 6440-58-0) was obtained from Unilong Industry Co. Ltd., located in China. Methylisothiazolinone and Methylchlorisothiazolinone (CAS No. 26172-55-4), were obtained from Choice Organo Chem LLP, located in India. The purified water obtained from an ultrapure water purification system manufactured by Millipore, located in the United States was utilized for several purposes, including the eluent as well as the preparation of standards and samples.

### Conditions for the chromatographic approach

The chromatographic studies were conducted using a Waters HPLC e2695 separation module system, which was equipped with Empower software and a differential refractometer (Model 2414, Waters, USA). The chromatographic separations were performed utilizing a packing material composed of silica particles that were coated with a copolymer comprising maleic acid and polybutadiene. The eluent chosen for the experiment was a solution consisting of 2.0% v/v formic acid dissolved in water. Table 1 presents a comprehensive enumeration of the technique parameters.

Table 1: HPLC method parameters overview.

No	Parameter	Aluminum Determination Method Parameters
1	Eluent	2.0% v/v formic acid in water
2	Diluent	2.0% v/v formic acid in water
3	Detector Type	HPLC coupled with Differential Refractometer (DRI) detector
4	Column	Alltech Universal Cation HR, (4.6 mm I.D. x 100 mm, 3 $\mu$ m, Grace division, Illinois)
5	Flow rate (mL/minute)	1.0
6	Column Oven Temp. (°C)	35
7	Sample Cooler Temp. (°C)	25
8	Injection Volume ( $\mu$ L)	10
9	Run Time in minutes	10
10	Elution Mode	Isocratic elution
11	DRI Detector attenuation (sensitivity)	64
12	DRI detector temperature (°C)	35
13	Polarity	+ (Positive)

### Standard solution

A standard solution of anhydrous aluminum chloride was made by dissolving the right amount in a diluent made of water with 2.0% v/v formic acid. This solution had a concentration of 0.25 mg/mL aluminum and was used to analyze the aluminum content in aldry lotion. The dependability of the aluminum peak response of the HPLC instrument was assessed by utilizing the Relative Standard Deviation (RSD) obtained from five distinct injections.

### Sample solution

In order to acquire the sample solutions of aldry lotion, a precise transfer of aldry lotion equivalent to 25 mg of aluminum was made into a 100 mL volumetric flask. Following the process of weighing, a volume of 70 mL of diluent was incorporated and subsequently subjected to sonication for a duration of 20 minutes with occasional shaking. Following the sonication process, it is recommended to let the specimen in the volumetric

flask drop to ambient temperature. The sample was diluted with a diluent to achieve the desired volume and was well mixed. The solution underwent filtration using a 0.45 $\mu$  Poly Vinylidene Fluoride (PVDF) syringe filter (Sterlitech, SKU no. 30VP045AN). Following the removal of the initial 4 mL of filtrate, the material was subsequently transferred into a HPLC autosampler vial with the intention of injection. The measured concentration of aluminum in the sample solution is 0.25 mg/mL.

### Hydrochloric acid solution preparation

In order to mitigate any potential interference caused by chloride in aluminum chloride on the retention time of aluminum, a solution of hydrochloric acid was created. This was achieved by dissolving 93 mg of hydrochloric acid with a concentration of 37% in 250 mL of diluent, resulting in a hydrochloric acid solution with a concentration of 0.13 mg/mL.

### Placebo of Aldry lotion

The precise formulation of aldry lotion remains undisclosed. Therefore, a mixture was prepared by combining approximately 5 grams of each of the following substances: 1,2-Propanediol, PPG-15 Stearyl ether, Cetearyl alcohol, Glycerol stearate, PEG-100 stearate, Urea, Laureth-23, Dimeticone, Sodium lauryl sulfate, DMDM hydantoin, Methylisothiazolinone, Methylchlorisothiazolinone, and Disodium EDTA. This mixture was then diluted with water to a total quantity equal to 100 grams.

### Stress studies

The utilization of stress testing can facilitate a more comprehensive comprehension of the degradation products of a medicinal product and the intrinsic stability of its molecule [13-23]. Therefore, both the sample and the placebo were subjected to varying levels of stress, as elaborated upon thereafter. The Aldry lotion composition underwent a stress test. The assessment of placebo interference, aluminum content, and mass balance was conducted on all samples subjected to stress in order to validate the stability-indicating nature of the reported approach. In order to subject the placebo and samples to thermal conditions, a water bath manufactured by Biolab Scientific Ltd., Canada (Model No. BBWA-101) with a temperature range of 5 °C-100 °C was utilized.

In order to subject the placebo and samples to thermal conditions, a water bath manufactured by Biolab Scientific Ltd., Canada (Model No. BBWA-101) with a temperature range of 5 °C-100 °C was utilized. All stress studies (alkali/ acid hydrolysis, thermal stress, oxidation, and photolytic) were performed by placing aldry lotion equivalent to 25 mg aluminum into individual 100 mL volumetric flasks. In the acid/alkali/ water hydrolysis/ oxidation stress experiments, 5 mL of 0.1N hydrochloric acid/ 0.1 N sodium hydroxide/ 0.5%(v/v) hydrogen peroxide solution was added to respective volumetric flask, and they were incubated in a water bath at a temperature of 60 °C for a period of 48 hours. In the case of thermal stress study, the aldry lotion contained volumetric flask was incubated in a water bath at a temperature of 60 °C for a period of 48 hours. To perform photolytic stress study, the aldry lotion contained volumetric flask was subjected to a minimum of 200 watts hours/ square meter of the ultraviolet region and 1.2 million lux hours of the visible region in a photostability test chamber (Model No. TP 200 G, Thermolab, India). Following the exposure, the solutions were subsequently cooled to the ambient temperature. About 70 mL of diluent was added to each volumetric flask. The solutions underwent sonication for a duration of 20 minutes with occasional shaking. Following sonication, the sample volumetric flasks were left to cool down to the ambient temperature. For acid/alkali hydrolysis, the solutions were neutralized using 0.1N sodium hydroxide / 0.1 N acid solution. The solution underwent filtration using a 0.45 $\mu$  Poly Vinylidene Fluoride (PVDF) syringe filter (Sterlitech, SKU no. 30VP045AN). Following the removal of the initial 4 mL of filtrate, the material was subsequently transferred into an HPLC autosampler vial with the intention of injection. The measured concentration of aluminum in the sample solution is 0.25 mg/mL. In addition to the sample solution, placebo solutions were prepared using the same process outlined above.

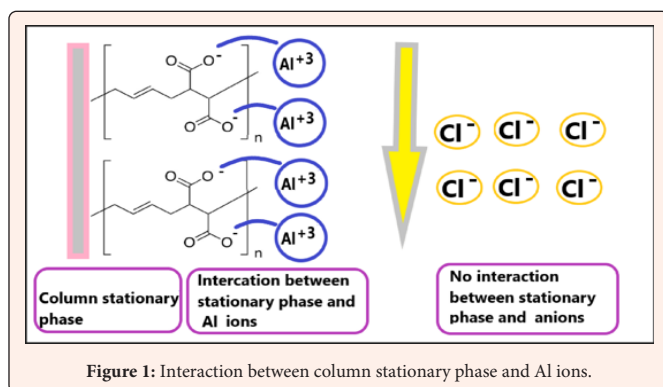
## Results and Discussion

### Overview of method development

The primary objective of the chromatographic approach was to effectively separate tightly eluting excipient and salt peaks from aluminum while ensuring that aluminum is eluted as a symmetrical peak that can be detected at low levels. Aluminum is an elemental metal that does not possess a chromophore capable of absorbing Ultraviolet (UV) radiation. Consequently, the selection of a Differential Refractometer Detector (RID) was made for the purpose of detecting aluminum [24-26]. The study involved examining

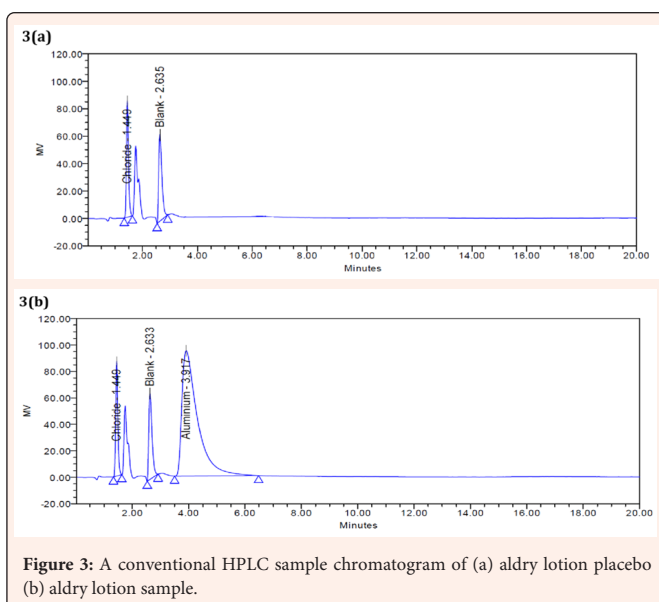
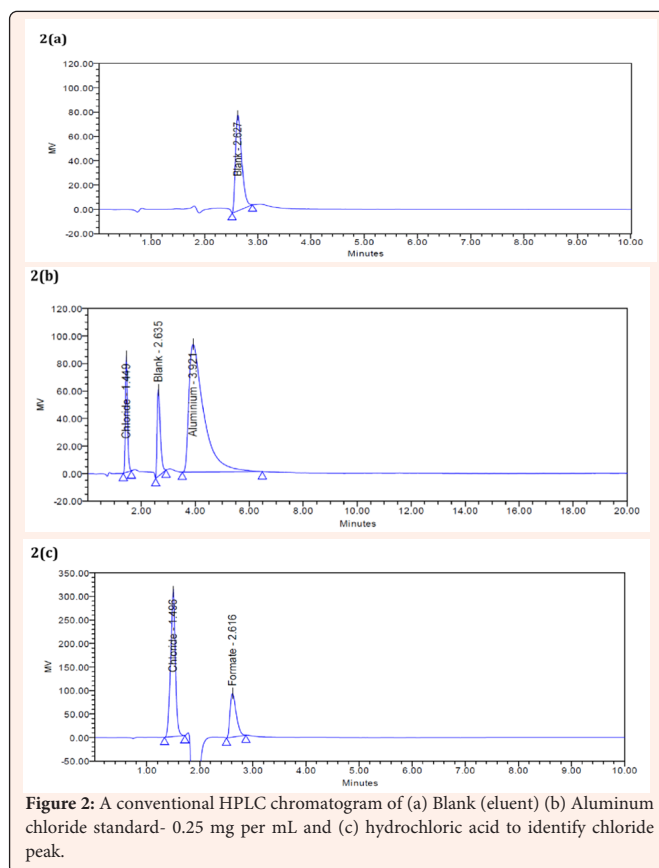
several levels of detector sensitivity to determine the most effective aluminum response while minimizing detector noise. The selected detector had a sensitivity value of 64. In order to mitigate fluctuations in refractive index, the temperature of the detector was maintained at a constant level of 35 °C, which closely matched the temperature of the column oven.

The cationic form of metal atoms will be observed in the salts. Aluminum, as an illustrative example, will be found in all aluminum chloride and aluminum chlorohydrate salts in the trivalent cationic state, denoted as  $Al^{+3}$ . A chromatographic separation was conducted using an Alltech Universal Cation HR column packed with a spherical silica coated with maleic acid/ polybutadiene co-polymer with dimensions of 4.6 mm in diameter and 100 mm in length. This column, obtained from the Grace division in Illinois, had a particle size of 3  $\mu m$ . The presence of polar-polar interactions facilitates the retention of cations in the injected sample due to the carboxyl functional groups ( $COO^-$ ) found in the maleate moiety. As the positive polarity of the metal atom increases, there will be a corresponding rise in its retention in the stationary phase. Trivalent metal ions have a higher degree of retention in comparison to divalent and monovalent cations. The chloride salt peak will elute early in the chromatography process due to their limited interactions with the stationary phase of the column, as illustrated in Figure 1.



The introduction of organic modifiers, such as acetonitrile and methanol, leads to the elution of aluminum in the void volume. Initially, experiments were conducted using different ratios of orthophosphoric acid and water, as well as different ratios of formic acid and water. The peak shape of aluminum was observed to be superior in formic acid eluents compared to orthophosphoric acid eluents. In addition, the augmentation of formic acid concentration in the eluent led to a decrease in retention time and an improvement in peak shape for aluminum. The eluent selected for this study consisted of a 2.0% v/v solution of formic acid in water. Several column temperatures (25 °C to 50 °C) were tested; however, when the column temperature crossed 40 °C, the resolution between aluminum and other salt peaks was deemed unsatisfactory. The temperature of the column oven was maintained at 35 °C.

The diluent used for sample and standard preparation was eluent, which consisted of 2.0% v/v formic acid in water. In order to mitigate any potential interference during the aluminum retention period, a blank eluent was introduced through injection. A single peak was seen in the chromatogram depicted in Figure 2(a), with a retention duration of 2.6 minutes. In the aluminum chloride standard, the elution of the aluminum peak occurred at a retention time of 4.0 minutes. Additionally, another peak was seen at a retention time of 1.5 minutes, distinct from the blank peaks (Figure 2(b)). In order to establish the association between the observed peak and chloride, an injection of a hydrochloric acid solution with a concentration of 0.13 mg/mL was conducted. The resulting chromatogram indicated the presence of a peak at a retention time of 1.5 minutes, which was subsequently proven to be representative of a chloride peak (Figure 2(c)). In addition, placebo of aldry lotion was injected and confirmed that the placebo has no interference at the retention time of aluminum peak in sample (Figure 3(a) & 3(b)). The diluent, placebo and chloride salt peaks are effectively separated from the aluminum peak in both the sample and standard solutions, given the selected chromatographic parameters. The aforementioned results substantiate the specificity of the methodology.



The system applicability of the approach was assessed to ensure its consistent and reliable performance, as well as its ability to produce precise measurement results. This study aimed to evaluate the system adaptability of the approach by conducting five injections of a standard concentration containing aluminum at concentration 0.25 mg/mL. The assessment of system appropriateness involved considering the percentage RSD of the aluminum responses obtained from the standard solution during the five repeat injections, as presented in Table 2. The RSD of the aluminum responses had a value below 2%, indicating a high level of accuracy and reliability within the system.

**Table 2:** Overview of System suitability results.

AlCl <sub>3</sub> Standard Concentration	Component Name	RT <sup>a</sup> (min)	% RSD <sup>b</sup>		Tailing Factor	
			Qualifying Limit	Perceived Value	Qualifying Limit	Perceived Value
Al-0.25 mg/mL	Aluminum (Al <sup>3+</sup> )	~4.0	< 2.0	0.8	< 2.5	1.9

<sup>a</sup>RT: retention time; <sup>b</sup>RSD - relative standard deviation of aluminum responses from five replicate injections of standard solution.

## Method Validation Summary

The range of an analytical method can be determined by analyzing data pertaining to precision, accuracy, and linearity [12]. The investigation into the linearity of aluminum was carried out using ten distinct concentrations, spanning from the 50% to 150% of the intended standard concentration (specifically, 0.125 mg/mL to 0.375 mg/mL). The obtained correlation coefficient (r) from the aluminum calibration plot exceeded 0.999, suggesting a linear relationship and a robust association between aluminum content and peak response. The results of the linearity test are presented in Table 3. The study on the accuracy of aluminum was conducted using four different concentrations, which spanned from the lower quantification limit 50% to 150% of the target aluminum content (0.25 mg/mL). These values ranged from 0.125 mg/mL to 0.375 mg/mL. The samples were generated by adding aluminum standard to placebo of aldry lotion at concentrations of 0.125 mg/mL, 0.25 mg/mL, and 0.375 mg/mL. The recovery of aluminum was seen to range from 97% to 103% at the 50%, 100%, and 150% levels. The accuracy of the procedure was proven by the findings presented in Table 3.

**Table 3:** Overview of accuracy and linearity results for aluminum.

Name	Accuracy; Recovery Range, %RSD			Linearity		
	50%	100%	150%	Range (mg/mL)	r	% Bias
	(0.125 mg/ mL)	(0.25 mg/ mL)	(0.375 mg/mL)			
Aluminum (Al <sup>3+</sup> )	98.5- 102.9%, 2.1	100.5- 102.3%, 0.8	99.9- 101.5%, 0.5	0.125- 0.375	0.9991	-1.2

The precision (repeatability) of the procedure was assessed by injecting six separate samples of aldry lotion. The assay of aluminum in aldry lotion was determined by employing the following formula. In this context, "B" represents the recorded response of the aluminum peak in the aldry lotion sample. "M" denotes the mean response of the aluminum peak obtained from five repeated injections of a standard solution. "W" signifies the concentration of aluminum chloride in the standard solution, measured in milligrams per milliliter. The molecular formula of aluminum chloride is AlCl<sub>3</sub>. The values "26.98" and "133.34" correspond to the atomic weight of aluminum and molecular weight of aluminum chloride, respectively. The symbol "F" is used to denote the weight of aldry lotion taken in milligrams (mg) for sample preparation. The variable "V" denotes the diluted volume in milliliter (mL). The molecular formula of aluminum chlorohydrate is Al<sub>2</sub>Cl<sub>3</sub>H<sub>2</sub>O<sub>4</sub>. The values "53.96" and "192.46" correspond to the molecular weights of aluminum and aluminum chlorohydrate, respectively. "L" is the labelled amount of aluminum chlorohydrate in the aldry lotion (20% w/w). The value is equivalent to 0.2. "D" is the density of the aldry lotion in mg per mL.

$$\% \text{ Assay} = \frac{B \times W \times 26.98 \times V \times 192.46 \times D \times 100}{M \times 133.34 \times F \times 53.96 \times L}$$

The observed Relative Standard Deviation (RSD) in the assay of aluminum was determined to be less than 2.0%. The aldry lotion containing aluminum had positive results within the range of 99.2-100.6%. The precision of the procedure was confirmed

by the findings presented in Table 4. The disclosed analytical approach for quantifying aluminum has a range of 0.125 mg/mL to 0.375 mg/mL, as determined through studies assessing accuracy, linearity, and precision (Table 3 & Table 4).

**Table 4:** Overview of method precision results.

Drug Product Name	Perceived Assay/ Recovery range (%)	% RSD <sup>a</sup>	
		Qualifying limit	Perceived Value
Aldry lotion	99.2-100.6	< 2.0	1.2

<sup>a</sup>RSD - relative standard deviation of aluminum recovery obtained from six sample preparations.

The robustness of the approach was assessed through intentional manipulation of chromatographic parameters, including variations in column oven temperature, flow rate, and formic acid concentration in the eluent. The evaluation of the key quality parameters of the approach, including the tailing factor, percentage of relative standard deviation, and retention time of aluminum was conducted under each robustness scenario. The impact of varying column oven temperatures within the range of 30°C to 40°C was investigated. The flow rate was altered to 0.9 and 1.1 mL/min in order to examine the impact of flow rate on the characteristics associated with quality. The impact of varying concentrations of formic acid in the eluent was investigated by utilizing 1.8% v/v and 2.2% v/v formic acid for the production of the eluent. The method's robustness is evidenced by the unaltered discrimination and performance under purposely altered testing settings. Table 5 is a summary of the robustness findings.

**Table 5:** Overview of Robustness results.

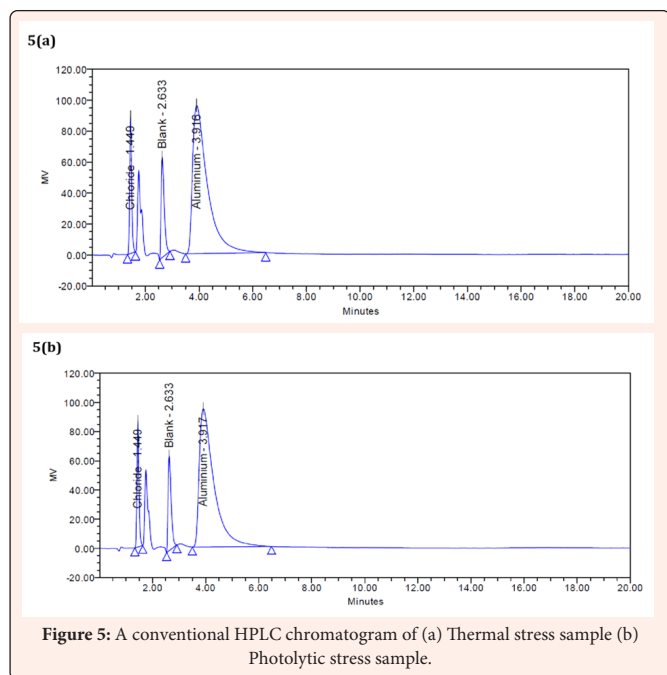
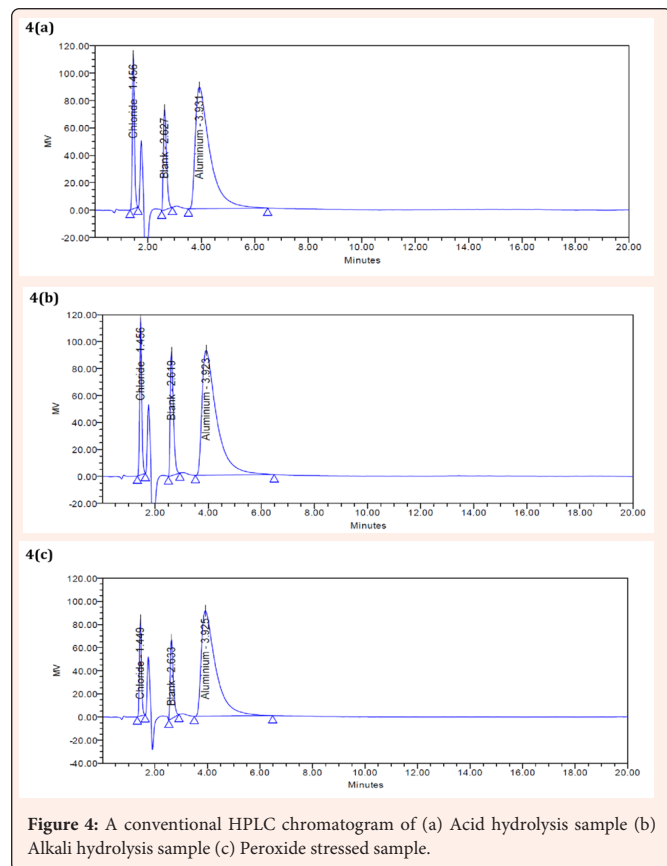
Robustness Parameter	RT <sup>b</sup> (min)	% RSD <sup>a</sup>		Tailing factor	
		Qualifying limit	Perceived value	Qualifying limit	Perceived value
Column temperature (30°C)	~4.0	< 2.0	0.6	< 2.5	1.9
Column temperature (40°C)	~3.8	< 2.0	1	< 2.5	1.8
Flow rate (0.9 mL/min)	~4.1	< 2.0	0.2	< 2.5	1.9
Flow rate (1.1 mL/ min)	~3.8	< 2.0	0.3	< 2.5	1.9
Formic acid (1.8%v/v)	~4.4	< 2.0	0.9	< 2.5	1.9
Formic acid (2.2%v/v)	~3.8	< 2.0	0.8	< 2.5	1.8

<sup>a</sup>RSD - relative standard deviation of aluminum responses from five replicate injections of standard solution; <sup>b</sup>RT: retention time

The stress investigation revealed no evidence of deterioration in the aluminum samples when subjected to alkali and acid hydrolysis, thermal, photolytic and oxidation (Figure 4 & 5). Regardless of the various stress conditions applied, the outcomes of the aluminum assay exhibit no significant deviation from those observed in the control sample. The differential refractometer detector is not capable of providing peak purity information. The lack of placebo interference seen during the retention time of aluminum indirectly validates the peak purity of aluminum. Through the process of superimposing stressed samples with equivalent placebo under each scenario, it was ascertained that there is no discernible interference stemming from the placebo. The findings of forced deterioration are summarized in Table 6. In order to get insight into the distribution of



impurities in stressed samples, the duration of the chromatographic run was extended to a total of 20 minutes. Nevertheless, no peak was observed following the elution of the aluminum peak. Therefore, a chromatographic run time of 10 minutes is deemed enough for the examination of aldry lotion samples.



**Table 6:** Overview of stress testing results.

Sample Name	Stress Condition	Aldry Lotion
		Assay of Aluminum (%)
Control sample	NA	100.9
Acid hydrolysis sample	0.1 N HCl-60°C/48 hours	100.0
Alkali hydrolysis sample	0.1 N NaOH-60°C/48 hours	100.4
Oxidation sample	0.5% H <sub>2</sub> O <sub>2</sub> -60°C/48 hours	99.1
Photolytic stress sample	UV-light region: 200 watt-hours/m <sup>2</sup> and visible-light region: 1.2 million lux-hours	100.7
Heat stress sample	60°C/ 48 hours	101.3

## Conclusion

The validation process shown that the disclosed technique exhibits satisfactory performance within the range of 0.125-0.375 mg/mL for aluminum concentration. The aforementioned characteristics encompass specificity, linearity, accuracy, precision, ruggedness, and robustness. During the stress testing, it was observed that aluminum did not exhibit any indications of degradation. The lack of placebo interference observed during the aluminum retention time under various stress settings indicates the purity of the aluminum peak and confirms the stability-indicating properties of the method being presented. The chromatography procedure has a duration of 10 minutes, and its sensitivity is similar to that of ion chromatography. The utilization of this technology in quality control (QC) departments proves to be straightforward for the purpose of monitoring the aluminum content in antiperspirant lotion, and aluminum raw materials.

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