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THIN-LAYER CHROMATOGRAPHIC ANALYSIS OF DYNAPAR-AQ INJECTION AFTER LIQUID–LIQUID EXTRACTION

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ABSTRACT

A simple and cost-effective thin-layer chromatography (TLC) method was developed for the separation and presumptive identification of the active constituent(s) of Dynapar-AQ injection following liquid– liquid extraction (LLE) and concentration [1,4,10,16]. The injectable formulation (1 mL) was diluted, acidified, and extracted using ethyl acetate (3 × 5 mL). The pooled organic phase was dried over anhydrous sodium sulfate, concentrated, and reconstituted in acetone according to established extraction and sample clean-up procedures [4,6,12]. Chromatographic separation was carried out on silica gel plates using two mobile phases: ethyl acetate : methanol : glacial acetic acid (8:2:0.1) and chloroform : methanol : acetic acid (9:1:0.1), selected based on previously reported TLC methodologies [1,3,5,10].

Visualization was achieved under ultraviolet light (254 and 366 nm) and by spraying with Dragendorff reagent [5,8,9]. The Dynapar standard and sample exhibited co-chromatographic behavior, producing a single major spot with reproducible Rf values, thereby supporting presumptive identification. The developed LLE–TLC method proved to be rapid, inexpensive, and reproducible, and is suitable as a screening technique in forensic and toxicological laboratories [11,16,19]. Methodological choices regarding solvent systems, extraction conditions, and visualization reagents were guided by established TLC and forensic extraction practices reported in the literature [1,3,4,10,16].

Keywords: Dynapar-AQ, thin-layer chromatography, liquid–liquid extraction, Dragendorff reagent,etc.

INTRODUCTION

Dynapar-AQ injection is a widely used pharmaceutical formulation containing diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID) belonging to the phenylacetic acid class. It is commonly administered by intramuscular or intravenous routes for the management of acute pain, inflammation, and postoperative conditions. Diclofenac sodium acts primarily through inhibition of cyclooxygenase (COX-1 and COX-2) enzymes, leading to decreased prostaglandin synthesis [13,14,15]. Despite its therapeutic utility, diclofenac has been associated with adverse effects and toxic manifestations, particularly in cases of overdose, improper administration, or misuse. From a forensic and toxicological perspective, the analysis of diclofenac in pharmaceutical preparations is relevant in cases involving suspected medication error, drug abuse, medico-legal investigations, and quality control disputes [11,12,18]. Therefore, the development of simple, reliable, and cost-effective analytical methods for its detection and screening remains important [20,21].

Because it is inexpensive, rapid, and capable of analyzing multiple samples simultaneously, thin-

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layer chromatography (TLC) remains a widely used screening method in forensic toxicology [5,10,11,16]. Following appropriate extraction from biological or pharmaceutical matrices, forensic laboratories frequently employ TLC for presumptive identification of pharmaceuticals and pesticides; solvent systems and visualization reagents are selected to optimize selectivity and sensitivity for the analyte class of interest [1,4,6,12]. Earlier TLC investigations on organophosphorus insecticides and other toxicants have demonstrated that careful selection of extraction procedures and mobile phases produces reproducible Rf values that allow reliable identification against standards [1,2,3,19].

A pharmaceutical formulation, Dynapar-AQ injection, was used in this study as a commercial sample. The objective of this work was to establish a validated and repeatable TLC methodology applicable in a forensic laboratory context and to modify conventional liquid–liquid extraction (LLE) and TLC techniques for the extraction and identification of the active constituent(s) of Dynapar-AQ from the injectable matrix [4,10,16,20].

MATERIALS AND METHODS

1. **Material and Reagent: Dynapar**-AQ injection (1 mL aliquots for analysis) — commercial vial, Dynapar reference standard (prepare stock) — (specify supplier / lot),Distilled water, Hydrochloric acid (dilute 0.1–0.5 N), Ethyl acetate (analytical grade) — extraction solvent, Anhydrous sodium sulfate — drying agent, Acetone — reconstitution solvent, TLC plates: silica gel G (glass / aluminium sheets), 60 F_{254} (Merck or equivalent), Development chambers, capillary tubes, glassware (separating funnel, conical flasks) [5,10,16]

   Visualization: UV lamp (254 and 366 nm) and Dragendorff reagent (for spray) [5,8,9]

   Other solvents for comparison runs: chloroform, methanol, glacial acetic acid

2. **Sample preparation and acidification**
   1. A 1.0 mL aliquot of Dynapar-AQ injection was pipetted into a clean glass vial.
   2. One milliliter of distilled water was added to reduce viscosity and facilitate mixing.
   3. One to two drops of dilute hydrochloric acid (0.1–0.5 N) were added to acidify the solution. Acidification facilitated the extraction of ionizable components into the organic phase where appropriate. [4,6,12]

3. **Liquid–liquid extraction (LLE)**
   1. The acidified solution was transferred into a 10–25 mL separating funnel.
   2. Five milliliters of ethyl acetate were added to the funnel.
   3. The mixture was shaken gently to avoid emulsion formation and was allowed to stand until clear phase separation occurred.
   4. The organic (ethyl acetate) layer was collected.
   5. The extraction was repeated twice more using fresh portions of ethyl acetate (5 mL each), and all organic layers were pooled. [1,3,4]

4. **Drying and concentration**
   1. Anhydrous sodium sulfate was added to the pooled organic extract and swirled until the solution became clear.
   2. The extract was filtered (or decanted) to remove the drying agent.
   3. The solvent was evaporated under gentle conditions using a rotary evaporator or a gentle nitrogen stream until near dryness.
   4. The residue was reconstituted in a suitable volume (0.5–1.0 mL) of acetone for application onto TLC plates. [4,6,12]

5. **TLC conditions**
   1. Silica gel G TLC plates were used as the stationary phase and were pre-washed and activated when required.
   2. The standard was applied as spots (1–2 µL per spot) using fine capillary tubes or a micropipette and allowed to air-dry.
   3. The plates were developed to a distance of approximately 8–10 cm. [5,10,16]
   4. The mobile phases evaluated were:
      a. Ethyl acetate : Methanol : Glacial acetic acid (8 : 2 : 0.1, v/v/v), used as a polar solvent system for separation of moderately polar analytes.
      b. Chloroform : Methanol : Acetic acid (9 : 1 : 0.1, v/v/v), used as a less polar solvent system commonly applied for aromatic or neutral analytes. [1,3,5,10]
   5. Prior to development, the chamber was saturated with mobile phase vapors for 20–30 minutes. The plate was then placed in the chamber and developed until the solvent

front reached approximately 8–9 cm, after which it was removed and air-dried. [5,10,16]

6. Visualization

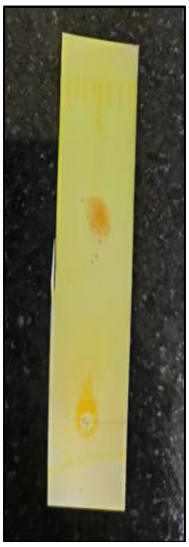
1. The developed plates were examined under ultraviolet light at 254 nm and 365 nm, and the positions of the spots were marked.
2. The plates were sprayed with Dragendorff reagent and allowed to develop color. The color intensity and appearance of the spots were recorded. [5,8,9,19]

RESULTS AND DISCUSSION

Table 1. Observed Rf values

Run	Solvent system	Standard Rf	Visualisation (UV/ Dragendorff)
1.	Ethyl acetate : Methanol : Acetic acid (8:2:0.1)	0.5686	UV 254nm, Dragendorff: [Orange]
2.	Chloroform : Methanol : Acetic acid (9:1:0.1)	0.7073	UV 365nm





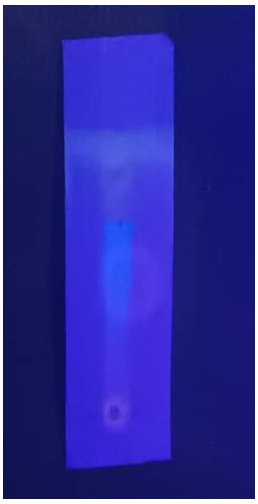


Figure. 1: Thin Layer Chromatography of DYNAPAR AQ after visualisation under UV Light at 254nm.	**Figure. 2:** Thin Layer Chromatography of DYNAPAR AQ after visualisation with Dragendorff reagent showing orange spot.	**Figure. 3:** Thin Layer Chromatography of DYNAPAR AQ after visualisation under UV Light at 365nm.

Thin-layer chromatographic analysis of Dynapar-AQ injection was successfully performed using two solvent systems. In ethyl acetate : methanol : acetic acid (8:2:0.1), the Dynapar standard produced a well-defined spot with an Rf value of 0.5686, which was clearly visible under UV light at 254 nm and developed an orange coloration with Dragendorff reagent [5,8,9]. In chloroform : methanol : acetic acid (9:1:0.1), a distinct spot was observed at an Rf value of 0.7073 under UV light at 365 nm, although no significant color reaction was obtained with Dragendorff reagent [5,10]. Both solvent systems produced good spot resolution with minimal tailing, and photographic documentation confirmed the reproducibility of the chromatographic separation [16,19].

Among the two solvent systems, ethyl acetate : methanol : acetic acid (8:2:0.1) provided superior visualization and sensitivity due to the positive Dragendorff response and optimal Rf range suitable for

identification [5,10]. The higher Rf obtained with chloroform : methanol : acetic acid (9:1:0.1) reflected greater analyte migration caused by the lower polarity of the mobile phase; however, reduced interaction with the visualization reagent limited its sensitivity [1,3,10].

The liquid–liquid extraction procedure using ethyl acetate, followed by drying and concentration, effectively isolated the active constituent from the injectable matrix, as evidenced by clear spots and absence of interfering bands [4,6,12]. Overall, the combined LLE–TLC approach proved to be a simple, rapid, and cost-effective screening method for Dynapar-AQ injection and demonstrated potential applicability in forensic and quality control laboratories [11,16,19]. Confirmatory analysis using instrumental techniques such as HPLC or GC–MS would be required for definitive identification and quantification [7,12,18].

CONCLUSION

For Dynapar-AQ injection, a simple LLE–TLC protocol was developed. Ethyl acetate extraction followed by silica gel TLC using ethyl acetate : methanol : acetic acid (8:2:0.1) and chloroform : methanol : acetic acid (9:1:0.1) produced repeatable and co-chromatographic spots for both the Dynapar standard and the sample, with a visible response to Dragendorff reagent where applicable [1,4,5,10]. The method was found to be suitable as a presumptive screening technique in forensic toxicology laboratories; however, confirmatory instrumental analysis was recommended for routine casework [7,11,18].

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